The Role of Ants in Seed Dispersal and Regeneration in a Degraded West African montane Forest-grassland Landscape, Ngel Nyaki Forest Reserve, Nigeria

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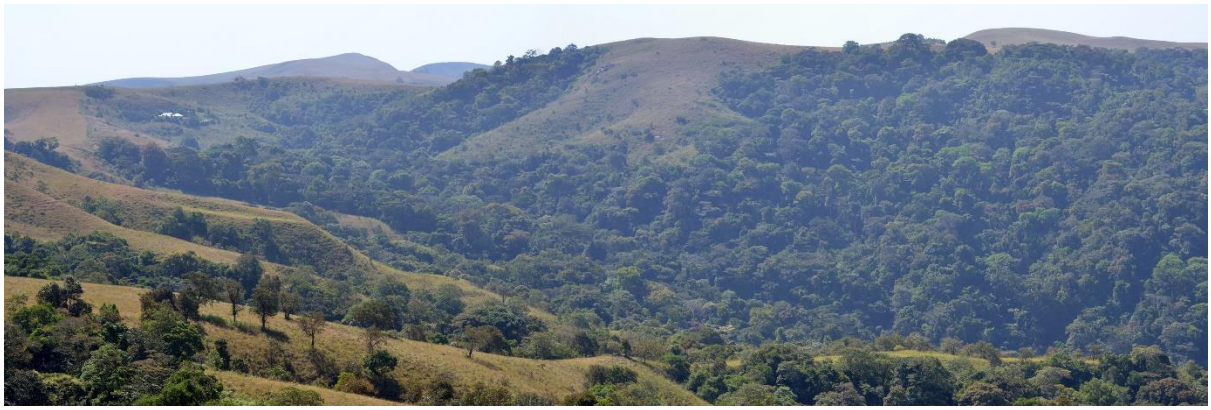

A thesis submitted in partial fulfilment of the requirements for the Degree of

DOCTOR OF PHILOSOPHY in ECOLOGY

In the University of Canterbury

By

Jennifer Arubemi Agaldo

University of Canterbury
Christchurch, New Zealand

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…think about the ant! Consider its ways and be wise.

Proverbs 6:6 (New International Readers Version).

Abstract

Beneficial interactions between ants and plant diaspores, such as the dispersal and cleaning of diaspores have been widely reported from the neotropical, subtropical and temperate regions of the world. Remarkably, there are no reports of such interactions from the Afrotropics. Hence, it is not known whether the nature and outcomes of such interactions in this region mirror those from other regions. Therefore, this study was undertaken to document the species, and functional trait diversity of ground-dwelling ants, and determine the contribution of interactions between ants, and diaspores, to regeneration in a West African montane forest-grassland landscape in Nigeria, which is ecologically diverse but has been under considerable anthropogenic pressure.

Ants were sampled from August 2015 to July 2016 using pitfall traps set along transects passing through forest, edge and grassland, to determine ant species richness and composition across the three habitats. Timed focal surveys at diaspore depots were conducted to record ant-diaspore interactions and an enclosure experiment in which vertebrates were excluded or allowed access to diaspore depots (with or without cages), was used to examine the effect of small, vertebrate competitors on the removal rate of diaspores of *Trema orientalis* by the two most abundant ant species. The effect of diaspore availability, size, texture, colour shade and chemical composition on ant-diaspore interactions were also investigated. Finally, the effects of manually cleaning seeds to mimic ant-cleaning, and sowing in soil taken from ants' nests on seed germination success, were investigated in nursery germination experiments.

Overall, ant species richness was low, with 17 species/morphospecies recorded across the three habitats. Species richness was higher in grassland and edge habitats, and species composition differed significantly between grassland and forest and between edge and forest, but not between grassland and edge habitats. Principal Component Analysis of functional trait values indicated trait diversity and adaptation of ant species to different levels of habitat complexity and resource exploitation. An unidentified *Pheidole* species dominated the forest samples while the grassland and edge habitats were dominated by *Myrmicaria opaciventris* and the *Pheidole* species from the forest samples. Only these two small, but abundant myrmicine ant species, interacted with diaspores of 10 plant species (moving six, and cleaning four); all non-myrmecochorous trees. Diaspores were cleaned on the spot or removed to ant nests/litter. Dispersal distances were low; ranging from 0.02 to 1.24 m with a mean of 0.29 ± 0.01 m. Diaspore size and texture significantly affected diaspore removal; ants only moved diaspores

that weighed ≤ 0.3 g and preferred soft seeds to hard ones. Larger diaspores were cleaned on the spot and hand-cleaned diaspores germinated significantly quicker than uncleaned ones. Soils from ant nests were significantly more porous and had higher moisture content than non-nest soils, but ants did not significantly alter soil pH, nitrogen, phosphorus and potassium content. Cowpea seeds sown in soil from ant nests grew faster compared to those in non-nest soil, suggesting that ant nests may provide better microsites for germination.

This first contribution on ant-diaspore interactions in an Afrotropical montane ecosystem, demonstrates that like in other regions, ants interact opportunistically, with non-myrmecochorous diaspores in potentially beneficial ways that could enhance seedling recruitment and regeneration in degraded habitats. But in contrast to those regions, the contribution of ants, to dispersal here, are minimal, reinforcing the reality that unless large vertebrate frugivores, which are the main long-distance dispersers in tropical forests are conserved, natural regeneration will be severely limited

Chapter 1: Introduction and Literature Review

1.0 Ants in terrestrial ecosystems

Ants belong to the insect order Hymenoptera. This order includes other social insect taxa such as bees and wasps, many of which are beneficial to man (LaSalle and Gauld, 1993). With about 13,000 named species and potentially an almost equal number of undescribed species (Bolton, 2012), ants are the most abundant and diverse social insects on Earth (Chomicki and Renner, 2017, Del Toro et al., 2012, Hölldobler and Wilson, 1990). They account for between 10-15% of the total biomass in most terrestrial ecosystems (Ellison, 2012, Folgarait, 1998, Hölldobler and Wilson, 1990, Zelikova et al., 2011). In addition to, and probably because of their numerical abundance and species diversity, ants exhibit a diversity of traits and interactions which enable them to provide important ecosystem services such as soil aeration, nutrient cycling, biological control of pests, pollination, and dispersal of the seeds of many plant species (Zelikova et al., 2011).

1.1 Ant-plant interactions

Interactions between ants and plants are extremely diverse (Beattie, 1985, Hölldobler and Wilson, 1990, Levey and Byrne, 1993) and occur across a wide range of Angiosperms (flowering plants) (Wilson and Hölldobler, 2005). These interactions can be antagonistic, mutualistic or, in some cases, both depending on the context in which the interaction occurs (Bronstein et al., 2006, Holland et al., 2005, Rico-Gray and Oliveira, 2007).

The main antagonistic ant-plant interaction is plant predation, expressed either as herbivory (in which ants feed on vegetative plant parts), or granivory, whereby they consume seeds (Andersen, 1987, Beattie, 1985, Hölldobler and Wilson, 1990). An indirect antagonistic interaction is when ants protect and tend aphids and scale insects that feed on plant sap and often transmit many pathogenic plant viruses (Gullan et al., 1993). Mutualistic ant-plant interactions include obligate ant-plant symbioses, and more opportunistic and facultative interactions such as when ants protect plants from other herbivores while nesting and feeding on the host plant

without causing significant injury (Heil et al., 2005, Iluz, 2011, Lach, 2003, Palmer et al., 2008). Another very important ant-plant mutualism occurs when ants act as dispersers of seeds of many plant species (Howe and Smallwood, 1982, Hughes and Westoby, 1992, Leal et al., 2007).

1.2 Seed dispersal

Seed dispersal is one of the most important ecosystem functions driving plant community dynamics (Garcia et al., 2010). It is the movement of viable units of dispersal - diaspores (fruits and seeds) away from parent plants (Nathan & Muller-Landau, 2000) to sites where they can germinate and be recruited as new plants (Gorb and Gorb, 2003, Howe and Smallwood, 1982, Hulme et al., 2002). It is an important step in plant reproduction, essential for the sustenance of plant communities (Howe, 1990), the colonisation of new areas (Dennis, 2007, Howe and Smallwood, 1982) and regeneration and restoration in both intact and degraded ecosystems (Wunderle, 1997).

Seed dispersal often occurs in multiple stages; referred to as phase I or primary dispersal, and phase II or secondary dispersal (Chambers and MacMahon, 1994, Vander Wall and Longland, 2004). In primary dispersal, seeds are moved from a parent plant through the agency of gravity, wind, ballistics, water, and/or animals such as birds and mammals, onto a surface, most likely soil or litter (Chambers and MacMahon, 1994). When facilitated by wind or long-distance vertebrate dispersers, primary dispersal is important for the colonisation of new areas (Howe and Smallwood, 1982), and the avoidance of distance and density-dependent antagonistic interactions with conspecifics and natural enemies (so-called Janzen-Connell effects), which are more intense close to parent plants (Janzen, 1970) and (Connell, 1971).

In the secondary phase of dispersal, diaspores are moved, usually over relatively shorter distances (Forget and Milleron, 1991), but this can complement primary dispersal and may provide the added benefit of directing such diaspores to microsites (directed dispersal hypothesis) which significantly influence germination success and eventual fate of seedlings (Wenny, 2001). Secondary seed dispersal systems are

however, widely variable because of the diverse potential combinations of dispersal agents (Nogales et al. 2007), which could include abiotic (e.g. water or wind or mechanisms) and biotic factors, such as scatter-hoarding rodents (Forget and Milleron, 1991, Vander Wall and Longland, 2004), and invertebrates e.g. dung beetles (Koike et al., 2012), and ants (Christianini and Oliveira, 2010, Lengyel et at., 2009, Levey & Byrne 1993; Pizo et al., 2005). It is this secondary dispersal phase involving ants, and its impact on regeneration in a West African montane forest, that forms the focus of this thesis.

1.3 Ant-mediated seed dispersal

1.3.1 Myrmecochory

Myrmecochory, ant-mediated seed dispersal, is the most extensively and well-studied mutualistic ant-diaspore interaction (Bronstein et al., 2006, Wunderle Jr, 1997). It is limited to seeds that possess a lipid-rich structure called an elaiosome (Lach et al., 2009), an adaptation to attract ants (Edwards et al., 2006). Such diaspores are moved to safe and suitable germination sites such as leaf litter and ant nests (Giladi, 2006, Gorb and Gorb, 2003) where the elaiosome is consumed (usually fed to larvae) and the seed is discarded unharmed, in nest galleries or waste piles outside the nest (Giladi, 2006, Gorb and Gorb, 2003). Myrmecochory is described as a facultative or "diffuse" mutualism because, while both partners derive some benefit (food for the ant and dispersal related benefits for seeds), there is often wide variation in the identity of the partners (Beattie, 1985).

Myrmecochory cuts across eco-regions and ecosystems; occurring in temperate forests of north-east America and Europe, dry sclerophyll vegetations of Australia, and fynbos of South Africa (Bronstein et al., 2006, Giladi, 2006) and the Neotropics (Horvitz and Schemske, 1986). Over one hundred ant species and about 11,000 plant species are involved in myrmecochory (Lengyel et al., 2010, Zelikova et al., 2011), which gives it enormous ecological significance (Beattie & Culver, 1981; Rice & Westoby, 1981; Handel et al. 1981; Milewski & Bond, 1982; Fenner, 1985; Petersen & Philipp 2001).

Several hypotheses have been proposed for the evolution and selective advantage to plants, of myrmecochory (Lengyel et al., 2009) and include: (i) predator avoidance as seeds removed quickly by ants to their nests, are able to avoid consumption by predators (ii) dispersal distance in which competition for seedling microsites is reduced by ant removal and movement of seeds significant distances away from unfavourable habitats directly under adults plant; (iii) directed dispersal in which seeds taken into ant nests are protected from fires common in some of the dry habitats where myrmecochory commonly occurs, and ant nests to which seeds are taken, are usually rich in some, or all essential plant nutrients (Lengyel et al., 2009).

1.3.2 Ants and non-myrmecochorous diaspores

Less well studied than myrmecochory, but gaining recognition due to its frequency of occurrence and potential ecological importance, is the phenomenon of interactions between ants and non-myrmecochorous diaspores, i.e. diaspores primarily adapted for dispersal by vertebrates (Gallegos et al., 2014, Passos and Oliveira, 2003). These interactions involve either the removal of diaspores of a wide variety of plants into leaf litter or ant nests (Passos and Oliveira, 2003) or seed cleaning, characterised by on-the-spot consumption of fruit pulp or diaspore arils (Christianini et al., 2007).

Such interactions are also considered to be diffuse mutualisms because they often involve numerous ant and plant species without evidence of specific adaptations. However, some ant species have been found to serve as key dispersers of particular plant species (Barroso et al., 2013). Generally, dispersal of non-myrmecochorous diaspores by ants is influenced by diaspore traits such as lipid content, diaspore size relative to ant size, seed shape and texture, and the presence or absence of appendages which provide a handle that ants use to transport the seeds (Christianini et al., 2007, Pizo and Oliveira, 2000, Retana et al., 2004).

1.4 Benefits of dispersal and seed cleaning by ants

The benefits of ant dispersal and seed cleaning for non-myrmecochorous species may include predator avoidance and/or directed dispersal; there is some evidence that deposition of seeds in ant nests provides predator avoidance (O'Dowd and Hay,

1980) and more favourable germination environments. However, it is difficult to differentiate ant-diaspore removal that is predation, from that which is dispersal, because some of the ant species involved are also seed predators (Arnan et al., 2012). Nevertheless, granivorous ants can play a dual role as predators and dispersers when they become satiated and fail to consume all collected diaspores (Arnan et al., 2012, Levey and Byrne, 1993, Retana et al., 2004). Such diaspores then have an improved chance to germinate away from other predators (Beattie et al., 2002, Crawley, 2000, Hulme et al., 2002). Diaspore cleaning by ants through the consumption of arils can enhance germination, and prevent seed mortality due to pathogen attack (Christianini et al., 2007, Ohkawara and Akino, 2005). These cascading effects potentially lead to increased seedling recruitment and regeneration in degraded habitat patches (Gallegos et al., 2014)

Studies provide variable support for the occurrence of the above-hypothesised benefits, but their relative importance appears to vary across habitats and regions and is linked to differences in the behaviour of the dispersing ant species (Chambers and MacMahon, 1994, Giladi, 2006). Notably, these hypotheses are based on studies limited to forests and savannas in the Neotropics, with a few in the Mediterranean, while the vast areas of Afrotropical forest remain unexplored.

1.5 The lack of studies on ant interactions with non-myrmecochorous diaspores in the Afrotropics

Afrotropical forests are known to harbour diverse ant (Fischer, 2012, Yanoviak et al., 2008) and plant communities (Mittermeier et al., 1999). Consequently, similar ant-plant interactions to those observed in the Neotropics are likely to occur but have never been reported. Therefore, their potential contributions to regeneration in African forests are currently unknown.

Ant-plant interactions may be especially important in Afromontane forests where there is a disproportionately high number of plants that produce small diaspores (Chapman et al., 2016). Furthermore, given the high rates of deforestation in such forests, which have led to significant habitat degradation and biodiversity loss

especially of vertebrate dispersers (Price et al 2011), such studies could provide useful insights into the contributions of secondary seed dispersers (such as ants), which may affect natural regeneration by determining the fate of many diaspores in such degraded ecosystems.

An opportunity provided by the Nigerian Montane Forest Project in Ngel Nyaki Forest Reserve, a degraded Afromontane forest in Nigeria, West Africa, was taken to investigate for the first time, interactions between ants and non-myrmecochorous diaspores in this region. The main objective was to identify ways in which such interactions influence the fate of diaspores with respect to dispersal distances, deposition sites, changes in soil properties within ant nests and the impact of this last factor on germination success. Results are interpreted and discussed with considerations for how they fit with observations and hypotheses derived from studies in the Neotropics

1.6 Afrotropical montane forests

Afromontane forests occur above elevations of 1500 - 2000 m. They are found along the East African Rift from the Red Sea to Zimbabwe, with the primary areas being the Albertine Rift Mountains of Uganda, Burundi, Rwanda, Democratic Republic of Congo, and Tanzania, the Ethiopian Highlands, and the Eastern Arc highlands of Kenya and Tanzania. Smaller areas include the Drakensberg range of southern Africa, the Cameroon Highlands, and Cameroon Line volcanoes in West Africa (Figure 1.1). Like montane forests in other tropical areas, Afromontane forests are often areas of high conservation priority owing to their high rates of floral and faunal species endemism (Brooks et al., 2001, Scharff, 1992, Tropek and Konvicka, 2010, White, 1981), as well as their recognised value as important watersheds and global hotspots of biodiversity (Price et al., 2011).

Figure 1.1: Afromontane Zones. I. West African and Cameroon highlands, II. Ethiopian and Arabian highlands, III. Western (Albertine) Rift, IV. Eastern Rift. V. Southern Rift, VI. Eastern Highlands, VII. Drakensberg (Andrew Z. Colvin (https://commons.wikimedia.org/wiki/File:Afromontane_Zones_(transparent).png), https://creativecommons.org/licenses/by-sa/4.0/legalcode).

1.7 Study location: Ngel Nyaki Forest Reserve

Within Nigeria, montane forest is restricted to a few locations in the east of the country bordering Cameroon; Southern Cross River (Oates et al., 2002), Gashaka-Gumti National Park, and the Mambila plateau, all part of the Cameroon Volcanic line within the Cameroon highland region (Chapman et al., 2004b). The focus of this study is Ngel Nyaki Forest Reserve, located on the western escarpment of the Mambilla Plateau (Figure 1.1).

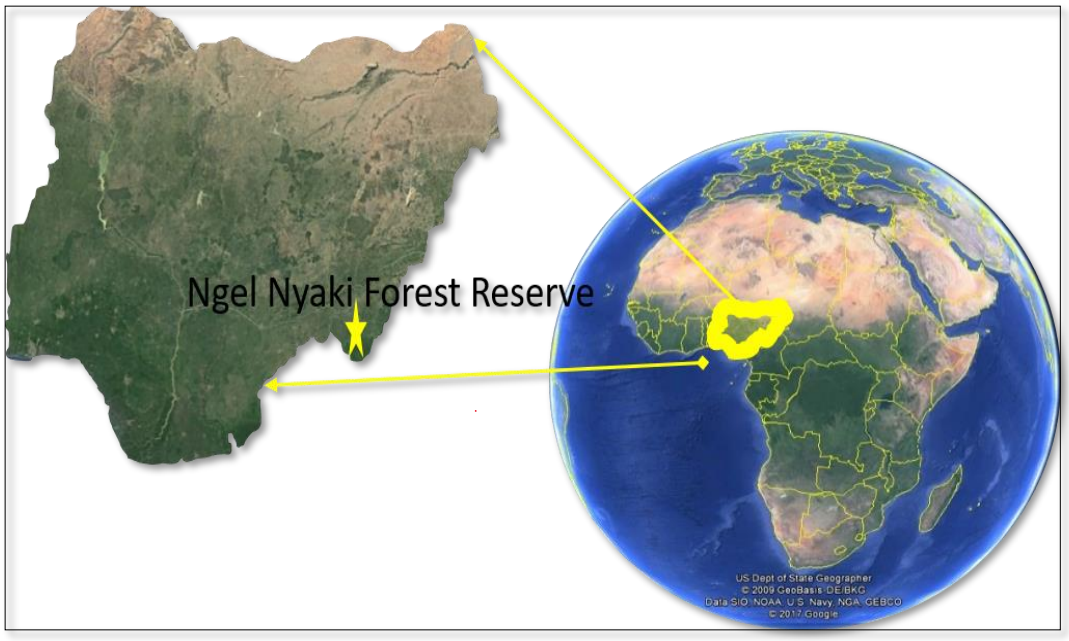

Figure 1.2: Africa showing the location of Ngel Nyaki Forest Reserve in South-eastern Nigeria. ©Google Earth

1.7.1 Conservation importance, degradation and restoration of Ngel Nyaki Forest Reserve, Nigeria

Ngel Nyaki Forest Reserve (07° 14' N, 011° 04' E), hereafter referred to as NNFR, lies at an elevation of 1400-1600 m. Its mean monthly temperature ranges from 13–26 °C for the wet season and 16–23 °C for the dry seasons, and it has a mean annual rainfall of 1800 mm (Chapman et al., 2004). It is home to an iconic montane forest with an especially diverse plant community that includes over 146 vascular plant species, some of which are near endemic to Afromontane regions (Beck and Chapman, 2008c, Chapman et al., 2004b, Chapman et al., 2001). It also has a rich and diverse fauna and is an IUCN important bird area (IBA) (Chapman et al., 2001). Species of conservation importance and priority include the endangered Nigeria-Cameroon chimpanzee *Pan troglodytes ellioti,* Civet *Civettictis civetta,* Putty-nosed Monkey *Cercopithecus nictitans martini,* different duiker species from the genus *Cepalophus,* and Buffalo *Syncerus caffer* (Beck and Chapman, 2008a, Chapman et al., 2001). Plant species on the IUCN red data list of conservation concern and

priority include *Pouteria altissima, Millettia conraui, Lovoa trichilioides* and *Entandrophragma angolense* (Chapman et al., 2001).

Within NNFR, which occupies an area 46 km ^{2} (Chapman et al., 2001), the area of forested escarpment is 7.2 km^2 (Beck and Chapman, 2008c) . The vegetation is a mosaic of forest escarpment and riparian forest surrounded by degraded grassland dominated by *Sporobolus pyramidalis*; a situation which has emerged following years of overgrazing and fire use by Fulani pastoralists (Barnes and Chapman, 2014, Chapman, 2008). The grasslands are characterised by the presence of small shrubs such as *Maesa lanceolata, Psorospermum febrifugum* and tall herbs such as *Ocimum basilicum, Guizotia* species and *Dissotis* species (Barnes and Chapman, 2014).

The high biodiversity within the reserve has led to its being commissioned as the site of the Nigerian Montane Forest Project (NMFP), a research project with a focus on forest conservation and restoration (www.afromontane.canterbury.ac.nz). However, like other forests in Nigeria, NNFR is threatened by anthropogenic activities, and over the last three decades insufficient protection has allowed increasing encroachment of human and cattle populations which has resulted in parts of reserve suffering from degradation arising from agricultural encroachment, hunting, fires and unsustainable cattle grazing practices (Chapman et al., 2004b).

The above activities have led to habitat loss and declines in populations of large mammalian and avian frugivores (Chapman, 2008), and is likely to have significant effects on plant demography and natural regeneration, because as is the case in most tropical forests at least 80% of the forest flora produce fleshy fruits that are adapted to dispersal by vertebrate frugivores (mostly birds and large mammals) (Chapman et al., 2004b).

The NMFP seeks to prevent further degradation and restore areas that have already been affected by degradation. In 2006, it launched an initiative to promote natural regeneration of degraded parts of the reserve that included the construction of a fence and fire breaks across the grassland area to exclude grazing, fire encroachment and other human-induced threats to protect the reserve from further degradation (Barnes, 2011, Barnes and Chapman, 2014). The project also has an active research

component that includes studies on seed dispersal mechanisms and that provides the opportunity and immediate context for the investigations in this thesis.

1.7.2 Animal-mediated seed dispersal studies in NNFR

Until approximately 50 years ago, NNFR was home to large vertebrate dispersers that included elephants and a viable population of chimpanzees. However, elephants are now extinct and only about 16 chimpanzees are left in the reserve (Beck and Chapman, 2008b, Beck and Chapman, 2008c). The loss and population decline of large vertebrate dispersers means that substitute dispersers are needed to sustain the plant community. Previous studies over the last decade have shown that primary animal seed dispersers in NNFR include primates, birds (Barnes and Chapman, 2014, Dutton et al., 2014), and rodents (Aliyu et al., 2014, Chapman et al., 2010). Fruit morphology has been shown to influence animal dispersal. For example, birds within NNFR are influenced by smaller seeds from red or blue fruits in drupes. Hence plant with such diaspores have more potential to be dispersed by birds compared to other seed types, probably due to the attractiveness of the colour to birds (Barnes and Chapman, 2014).

As part of an investigation into post-dispersal seed predation and secondary dispersal, and other ecological interactions that might foster natural regeneration in NNFR, ants were opportunistically observed interacting with some fruit and seed species (Chapman, *pers, Comm*.; Roseli, 2014). This stimulated interest to investigate ant-diaspore interactions in more detail and determine their ecological role within the reserve. It is expected that the community of ants within NNFR will differ between the core forest and degraded grassland because of structural differences related to the plant species present within these habitats, and this is expected to affect the nature and frequency of ant-diaspore interactions across these habitats.

Figure 1.3: Ngel Nyaki Forest Reserve showing core forest and surrounding degraded grassland. ©Google Earth

While the core forest and grassland are distinct habitats that can influence ant-diaspore interactions within NNFR (Figure 1.3), these two habitats are linked by the forest edge. In fragmented habitats such as NNFR, the edge habitat is a part of the forest (Murcia, 1995). Understanding how ants interact with seeds within this habitat could shed some light on the possibility of plant succession occurring from the core forest into the grasslands since edges can serve as doorways through which forest regeneration occurs (Holl and Lulow, 1997).

The current study of ant-diaspore interactions in NNFR will provide a better understanding of what ecosystem services they provide and specifically, if/how they affect seed dynamics and regeneration within the area. Such information is crucial if ecosystem functions such as seed dispersal and natural regeneration are to be enhanced for restoration purposes (Fonseca and Ganade, 2001). Furthermore, in light

of studies in the Neotropics that have demonstrated the frequency and significance of such interactions, it would be of ecological interest to determine whether the patterns observed in the Neotropics are mirrored in the Old World.

1.8 Aim of study and research questions

The overall aim of this thesis is to investigate interactions between ants and diaspores in NNFR and the potential of such interactions to contribute to the regeneration of the degraded grassland and forest in this West African montane habitat. Specifically, it seeks to answer the following questions:

(i) What ant species are present in NNFR and what foraging guilds and functional groups do they represent?

(ii) Do ants interact with diaspores and what species of ants and plants are involved?

(iii) Are there differences in the number and frequency of interaction between core forest (forest), forest edge (edge) and in the grassland?

(iv) What seed traits (size, shape, weight, presence or absence of elaiosomes) affect/influence these interactions?

(v) Do the seeds taken by ants end up in better/more suitable germination sites than others? What is the mean dispersal distance of ant-dispersed diaspores and does this vary across habitats?

(vi) What effect do ant-diaspore interactions have on seed germination and seedling recruitment?

1.8.1 Thesis outline

I used four main data chapters (2-5) to answer my research questions. Although each chapter is written as a stand-alone paper (to be published), they are connected in

addressing the overall aim of the thesis. Relevant background literature to each data chapter is provided with some repetition of some themes.

In Chapter 2, I assessed ant species richness and functional trait composition in the three main habitat types; grassland, edge and forest habitats. This was important to know because I expected that ant-diaspore interactions across habitats would be affected by the abundance and diversity of ant species as well as their functional groups or traits.

In Chapter 3, I investigated ant-diaspore interactions; I identified the ant species interacting with diaspores, the plant species they interacted with, and the nature of interactions. I was particularly interested in whether ants dispersed diaspores.

Factors that influence ant-diaspore interactions were investigated in Chapter 4. Diaspore availability was assessed across seasons, spatial and temporal variation in foraging activity of ant species interacting with diaspores was also assessed. Morphological and chemical traits of diaspores were measured to investigate traits influencing ant interactions. Predation by other competitors such as rodents relative to removal by ants was also assessed. The results provide some insights into how these factors that might influence/enhance ant-diaspore interactions.

Some seeds are surrounded by an aril, a fleshy cover of a seed with nutritional value (Cordeiro and Howe, 2003). Ants may clean seeds by consuming the aril (Ohkawara and Akino, 2005, Oliveira et al., 1995, Pizo and Oliveira, 1998). The focus of Chapter 5 is an investigation into seed cleaning of the arillate species *Paullinia pinnata*. Specifically, I wanted to know if, and if so how, seed cleaning influenced seed germination rates and subsequent recruitment of seedlings. To do this I compared germination rates of cleaned diaspores mimicking ant exploitation with uncleaned diaspores in germination experiments. Also in Chapter 5 I explored how ants affected soil physical and chemical properties in their nests and how this and influenced plant growth.

Chapter 6 synthesises the main findings from preceding chapters. It evaluates whether overall, ants can contribute to regeneration in NNFR and places results in

the wider context of ant-diaspore interactions and their ecological role given massive declines in large bodied vertebrate dispersers.

Chapter 2: Species and functional trait diversity of ground-dwelling ants in a West African montane forest

Abstract

Ants are the most diverse and dominant taxonomic group in many terrestrial ecosystems where they play many important roles. Due to their abundance, high diversity, and ubiquity, they are suitable for studying ecosystem function. In this study, I investigated ant species richness and evaluated the functional traits of the species encountered to understand their potential contributions to the regeneration of a degraded montane grassland-forest landscape in West Africa. Pitfall traps were used to collect ants in grassland, edge, and forest habitats at the Ngel Nyaki Forest Reserve (NNFR). Collected samples were identified to species level where possible, and 11 morphological (functional) traits relating to the ecological role of worker ants were taken from 5-10 individuals of each collected species. In total, 2709 individual ants from 17 species/morphospecies in 12 genera, were collected. Species diversity index was low (1.41). Ant diversity index was highest for grassland habitat (1.60), followed by edge and forest habitats, which had diversity index of 1.23 and 0.40 respectively. Season alone did not affect ant species richness but the interaction between year and season affected ant species richness, while the interaction between season and minimum temperature did not effect ant species richness. Two dimensions of a principal component analysis (PCA) explained 83.7% of the variations in ant functional traits. Ant species that occurred both in grassland and edge habitats were mostly omnivorous and had more variation in functional traits compared to ants which occurred exclusively in edge and forest habitats where much less variation was observed. Like most montane landscapes, ant species richenss and diversity in NNFR was generally low, perhaps due to lower temperature and productivity associated montane forest regions. The variation in functional traits among ants occurring in the grassland and edge habitat suggests that the impact of ants on ecosystem functions in these habitats could more significant.

2.1 Introduction

Biodiversity is essential for ecosystem persistence and stability because it provides the basic elements for species interactions within food webs and ecosystem processes (Cardinale et al., 2012, Chapin et al., 2000). There is therefore, much concern over how recent massive losses in biodiversity (caused by rapid environmental, anthropogenic and land-use changes) will affect ecosystem function and stability (Foley et al., 2005, Newbold et al., 2015). Most affected by biodiversity loss are tropical forests, which harbour the majority of global terrestrial biodiversity (Slik et al., 2015) and yet remain under constant pressure from deforestation, fragmentation, and selective logging (Malhi et al., 2014, Myers et al., 2000).

There are strong links between species richness and ecosystem functioning, so that declining diversity often leads to decreased ecosystem functioning (Cardinale et al., 2006, Loreau et al., 2001, Schwartz et al., 2000). Recent studies however, reveal that the relationship between biodiversity and ecosystem function is complicated and context-dependent and that taxonomic (species) diversity may not always translate to increased functional diversity and ecosystem functioning (Naeem and Wright, 2003). These studies have taken a trait-based approach which enable generalisation across species (McGill et al., 2006), rather than one based solely on taxonomic species richness.

This trait-based approach is predicated on the assumption that the effects of species on ecosystem functioning depend on their morphological, chemical, physiological or behavioural traits (Hooper et al., 2005, Symstad et al., 1998). This has greatly increased understanding of what components of diversity are important for ecosystem functioning and stability (Mayfield et al., 2010, Naeem and Wright, 2003). It has been demonstrated that focusing on the traits of species within a community enables more accurate predictions of what the loss of those traits would mean for the ecosystem in which they function (Cadotte et al., 2011, Hooper et al., 2005). Hence, conservation efforts aimed at preserving and restoring degraded ecosystems should include studies of species assemblies as well as their functional traits, to ensure that such efforts are more effective in restoring ecosystem function.

2.2 Ant diversity and function in terrestrial ecosystems

Ants as a taxonomic group, are particularly suitable for the study of diversity and ecosystem functioning because of their ubiquity, abundance, species richness and diversity (Hölldobler and Wilson, 1990). They are social insects in the order Hymenoptera, and all belong to a single family Formicidae (Folgarait, 1998). With 307 genera and 21 subfamilies which hold about 13000 species and an estimated equal number of undescribed species (Bolton, 2012), ants are the most diverse group of social insects on Earth (Chomicki and Renner, 2017, Del Toro et al., 2012, Hölldobler and Wilson, 1990). Majority of ant diversity is however, found within four main subfamilies: Myrmicinae (~ 6000 species), Formicinae (~ 3000 species), Ponerinae (~1100) and Dolichoderinae (~ 700 species), which account for over 87% of all described ant species (Guénard, 2001).

Given their diversity, it is not surprising that ants have developed diverse foraging strategies, which bring them into interactions with both closely and distantly related taxa (Boulton et al., 2005). They forage as generalist predators on a wide range of arthropods, as scavengers, omnivores, and collectors of exudates such as nectar from flowers and sap from plants (Andersen, 1995, Carrol and Janzen, 1973); competing with other animal taxa for these resources (Baur et al., 1998, Carroll and Janzen, 1973, Detrain and Deneubourg, 1997, Human and Gordon, 1997). Through their foraging activities, ants may control pests (Garrido et al., 2002), or consume and disperse the seeds of many plant species, (Christianini et al., 2007, Christianini and Oliveira, 2009, Leal et al., 2007, Youngsteadt et al., 2009), which can in turn, affect seedling recruitment and natural regeneration (Gallegos et al., 2014).

Additionally, through nest making on, and below-ground activities, ants can improve the physical and chemical properties of soils (Wardle et al., 2011), and significantly affect sequestration of below-ground carbon and biogeochemical nutrient cycling, which impact entire habitats rather than just their nest locations (Folgarait, 1998, Wardle et al. 2011).

The above attributes and functions, underlie the ecological dominance and significance of ants in many terrestrial ecosystems (Boulton et al., 2005, Fittkau and

Klinge, 1973, Herrera and Pellmyr, 2009, Majer, 1990, Rico-Gray and Oliveira, 2007).

2.2.1 Functional trait diversity in ants

The fact that ants are very speciose and perform so many functional roles suggests that they possess diverse functional traits (Petchey and Gaston, 2006, Walker et al., 2008). The possession of diverse functional traits contributes to functional diversity at the community level and is important because it determines how strongly species can affect various ecosystem functions (Bihn et al., 2010, Tilman et al., 1997). Based on shared functional traits, species may be classified into functional groups; sets of different species (taxa) that interact in similar ways with ecosystem processes (Bonsdorff and Pearson 1999). Thus, habitat modifications, such as disturbance or land use change that affect species richness and abundance, are also likely to lead to changes in functional groups and alterations of community functional diversity (Tilman et al., 1997). Therefore, identifying the foraging strategies of ant species within communities in specific habitats and grouping them into foraging guilds, may allow us to predict their functional roles in such habitats. So for example, ants that feed on a wide range of arthropods as can be considered to be generalist predators, while those that feed widely on plant and animal material are categorised as generalist omnivores.

Functional trait variation or diversity within and among biological communties has been linked to features of the physical environment, which act as environmental filters that either allow, or filter out species based on whether or not they possess the traits needed to persist within the particular set of conditions created by the filters (Wiescher et al., 2012). Thus, complex habitats that present a wide range of environmental conditions and niches, are likely to support, or lead to more trait variation than those with narrower ranges. In addition, biotic factors, such as the presence of dominant and aggressive species that intensify competitive interactions, may also act in combination with environmental filters to determine what species and therefore, what traits, are present within community assemblages (Petchey and Gaston, 2006). Consequently, habitat modifications, such as disturbance or land use changes that affect species richness, composition, and abundance, are likely to lead

to changes in functinal groups and community functional diversity (Tilman et al., 1997).

2.2.2 Ants and environmental monitoring

The ability of ants to affect all trophic levels makes them suitable for monitoring the impacts of environmental and anthropogenic change on ecosystems (Andersen, 1995). Because ants are relatively sensitive to changes in the environment (Andersen and Majer, 2004, McGeogh, 1998), disturbance within a habitat often leads to a shift in species composition (Zelikova, 2008). Hence their presence or absence can usually be linked to certain measurable environmental conditions (Brown, 1997, McGeogh, 1998). They are also able to ecolonise disturbed areas earlier than other taxa (Jackson and Fox, 1996, Vasconcelos, 1999), especially when the source of disturbance has been eliminated (Folgarait, 1998). This makes ants useful in assessing the success of restoration of degraded landscapes (Andersen and Sparling, 1997, Wunderle Jr, 1997). However, the lack of baseline data on ant species and other arthropods in many areas limits their use as bioindicators (Longcore, 2003).

2.3 Regional and local patterns of ant species richnes and diversity

Although ants are present in most terrestrial habitats across biogeographical regions, there are local and regional differences in their species richness and abundance. (Folgarait, 1998, Guénard, 2001). These disparities are strongly linked to regional and latitudinal variations in climate (Dunn et al., 2009). Globally, ant diversity is highest within the warm tropical regions close to the Equator, and declines with increasing latitude in both northern and southern hemispheres (Hölldobler and Wilson, 1990). However, in the northern hemisphere, declines in ant diversity along latitudinal gradients are greater, compared to the south, mainly due to warmer temperatures and more equable climate (Dunn et al., 2009). Also within the southern hemisphere, there is greater ant diversity in Australasia and the Neotropics (where several countries or provinces are known to harbour more than 80 genera) compared to similar latitudes in the Afrotropics (Guénard et al., 2012).

At local scales, patterns of ant diversity can be observed, and habitat characteristics such as disturbance and elevation, are major factors affecting local ant diversity

(Folgarait, 1998, Gibb and Hochuli, 2002, Kwon et al., 2014). Graham et al. (2004) for example, showed that highly disturbed habitats had fewer ant species than undisturbed or moderately disturbed areas; with one, or a few ant species dominating highly disturbed areas (Graham et al., 2004).

2.3.1 Elevational gradients and ant diversity- ants in montane forest habitats

Plant and animal assemblages are known to change with increasing elevation, (e.g. Bruijnzeel and Proctor, 1995; Chapman et al., 2016) and studies show that tropical montane (high elevation) forest habitats, differ from lowland forests in faunal/floral composition and diversity, and are characterised by relatively high levels of species endemism compared to lowland habitats (Myers et al., 2000). As with latitude, ant species richness declines with elevation (Table 2.1) and ant communities generally differ in composition and diversity along elevational gradients, with fewer ant species at higher elevations or in montane forests compared to lowland forests (Peterson et al., 1993, Wikramanayake, 2002).

While most studies have observed a continuous decline in species richness, with the maximum species richness found in lowland habitats, others have detected maximum richness at mid-elevation (Sanders, 2002). In both cases, at elevations > 1500 m, species richness tends to decrease sharply with increasing altitude. Within tropical latitudes, several studies have estimated the upper limit of ant distribution to be approximately 2500 m (Wilson, 1958; Bruhl et al., 1998; van der Hammen and Ward, 2005; Malsch et al., 2008).

Table 2.1: Ant species richness along elevational gradients in some tropical montane forests

Location	Altitude (m)	Sampling method	No. of species	Reference
Montane region (KwaZulu-Natal)	1720 -1900	P	28	(Kotze and Samways, 1999)
Mt. Kinabalu, Sabah, Malaysia	560	W, P	128	(Brühl et al., 1999)
,,	800	W, P	106	,,
,,	1130	W, P	58	,,
,,	1360	W, P	26	,,
,,	1530	W, P	27	,,
,,	1740	W, P	9	,,
,,	1930	W, P	11	,,
,,	2025	W, P	6	,,
,,	2300	W, P	3	,,
Kakamega forest, Kenya	1550	W, P	329	(Fischer, 2012)

P= Pitfall trap; W = Winkler leaf litter extraction; B = Beating Tray

Ant species composition and trait diversity, as well as the abiotic and biotic factors affecting these indices, remain poorly assessed in most Afrotropical landscapes; Guénard et al (2012) for example, assessed global patterns of ant diversity and showed that parts of West and Central Africa are among leading regions with a poorly explored ant fauna. Consequently, current estimates of ant species richness in the Afrotropical region probably reflect poor documentation and likely represent a fraction of actual diversities and species richness in Africa. This view is supported by the findings of Guénard et al (2012) based on extrapolations from current data, which suggest that poorly explored regions contain many undiscovered ant taxa, which may however, be lost due to high levels of habitat degradation and loss. Based on this, they recommend that poorly explored areas be prioritized for exploration and conservation (Guénard et al., 2012).

Inventories of ground dwelling species assemblages and assessment of their species richness and trait diversity is an important step in addressing the poor state of current knowledge and may provide insights on how these relate to the functional roles of ants in these areas. Such information would be especially valuable in assessing or predicting how ants affect the success of restoration activities in degraded ecosystems (e.g. Jansen, 1997, Longcore, 2003).

2.4 Nigerian Afromontane forest: Ngel Nyaki Forest Reserve

Ngel Nyaki Forest Reserve(NNFR) in Taraba State Nigeria, forms part of Nigeria's most important and floristically diverse submontane forest habitat (see Chapter 1 for detailed description), and its ecological importance has been recognised by local and international ecologists and conservationists.

Despite its importance, NNFR faces significant threat from anthropogenic activities, and has over the last three decades, suffered degradation arising from agricultural encroachment, fires, and unsustainable cattle grazing (Chapman et al., 2004a) with a cascade of negative consequences; including declines in populations of large-bodied mammalian and avian frugivores that are important seed dispersers (Chapman, 2004, 2008). Consequently, conservation of the fauna and flora, and restoration of degraded areas of the Reserve have been made a priority through a collaboration between the Taraba State Government and the Nigerian Montane Forest Project (NMFP).

To ensure the success of this venture, furthering our understanding of the ecological components and processes that may contribute to regeneration in NNFR is crucial. Identifying ground dwelling ants, the pattern of variability in their functional traits, and the functional groups they represent in NNFR, would allow us to predict their impact on various ecological processes- including seed dispersal and regeneration- which is the central focus of this thesis.

2.5 Aim

This chapter explores species richness, composition, and functional trait diversity of ground-dwelling ant species in Ngel Nyaki Forest and its surrounding grassland. It seeks to provide a preliminary list of ground dwelling ant species that occur in

NNFR, and based on their functional traits, discuss how they may contribute to regeneration in this degraded habitat. The inventory of species generated, and their associated functional traits is expected to provide the foundation for hypotheses and further studies on the ecological role of ant species and communities in this environment, with particular regard to regeneration and restoration efforts.

The specific objectives of this chapter are to:

1. Determine community composition and spatio-temporal variations in species richness and diversity of ground dwelling ants across three habitat types (grassland, forest edge, and forest) in NNFR

2. Identify variation in foraging guilds (predators, generalists, omnivores, and carnivores) across major habitats types (grassland, forest edge, and forest) in the NNFR.

3. Identify sources of variation in functional traits of ant communities in the NNFR and identify species driving such variations.

The null hypotheses were as follows:

- Ant species composition and richness will not vary across habitat types.

- There will be no variation in ant functional traits of in the forest/grassland matrix of NNFR.

2.6 Methods

2.6.1 Study area- Ngel Nyaki Forest Reserve(NNFR)

This study was undertaken across three sites within NNFR. Sites were separated by a distance of at least 0.5 km, and represent a gradient from core forest to previously overgrazed grassland (Figure 2.1). The three sites were individually fenced-off about ten years ago to exclude grazing and fire to enhance natural regeneration in the grassland (Roselli, 2014). For this study, two 320 m-long transects were established 20 m apart in each site, and extended from the regenerating grassland into the forest core.

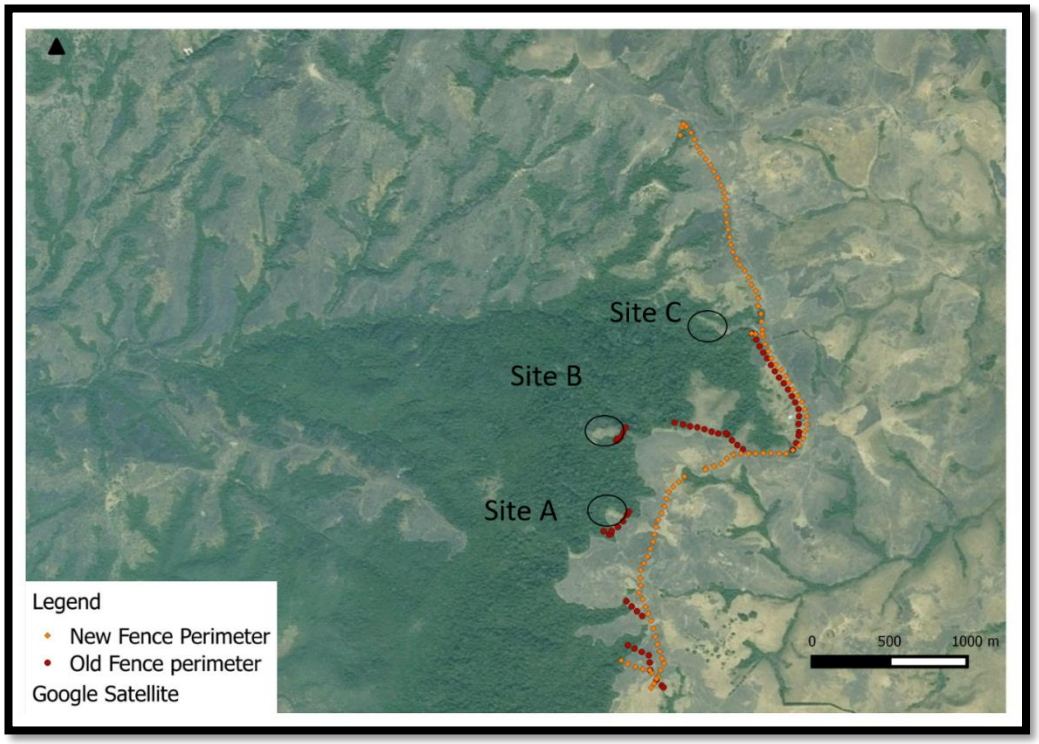

Figure 2.1: Map of Ngel Nyaki Forest Reserve showing study sites. Adapted from Google Maps.

2.6.2 Sampling and sorting of ants

To assess ant species composition within core forest, edge, and degraded grassland habitats, ants were sampled every month, at each site for 10 months (August 2015 and July 2016) encompassing both wet and dry seasons. Sampling was carried out using pitfall traps placed at 13 points on each transect beginning at the forest edge (0 m from the outermost forest canopy) and going in opposite directions into the forest and grassland at 5 m, 10 m, 20 m, 40 m, 80 m and 160 m (Figure 2.2) after the method of (Barnes, 2011, Kotze and Samways, 1999, Kotze and Samways, 2001). This design was used to compare ant species composition across the three habitat

types.

Figure 2.2: Experimental set up along forest to grassland gradient showing positions of pitfall traps.

Each trap comprised of a plastic cup with a rim diameter of 6.5 cm and depth of 8 cm. A funnel with the narrow end cut off was inserted over the opening of the trap to prevent agile ants from escaping (Millar et al., 2000). Between 1-2 inches of water containing a drop of detergent to break surface tension, was poured into each trap to retain trapped insects (Laub et al., 2009). Holes for the pitfall traps were dug in the ground a week before sampling began to avoid an effect on the trap catch due to disturbance caused by digging (Greenslade, 1973). Traps were placed in previously dug holes with the upper rim of the cup, level with the soil surface (Ipser et al., 2004). Traps were set between 0900 and 1100 hours and operated for 24 hours to allow collection of species active at different times of the day.

All ants caught in traps were removed and rinsed with clean water to remove the soapy water residue (Laub et al., 2009). They were then sorted per trap collection, and preserved in 70% ethanol in labelled tubes. Samples were identified in the laboratory in the School of Biological Sciences, University of Canterbury. Ant identifications were based on their external morphology, observed under a Wild A. S.

410 dissection microscope (at x40 magnification) and guided by taxonomic keys . Generic identifications were checked and confirmed by an expert ant taxonomist, Dr Rodrigo M. Feitosa, who separated the specimens into morphospecies and species where possible.Voucher specimens were deposited in the Entomological Collection Padre Jesus Santiago Moure (DZUP), at Universidade Federal do Paraná, Brazil.

2.6.3 Determination of slope at the study sites.

Slope was determined at 13 points on each transect by placing a metre rule horizontally on the ground, and measuring the adjacent distance (in metres), at right angles to the metre rule (Figure 2.3). A plumb line was used to ensure that the meter rule was horizontal to the ground. Slope was calculated as the vertical distance from the ground divided by 1 m.

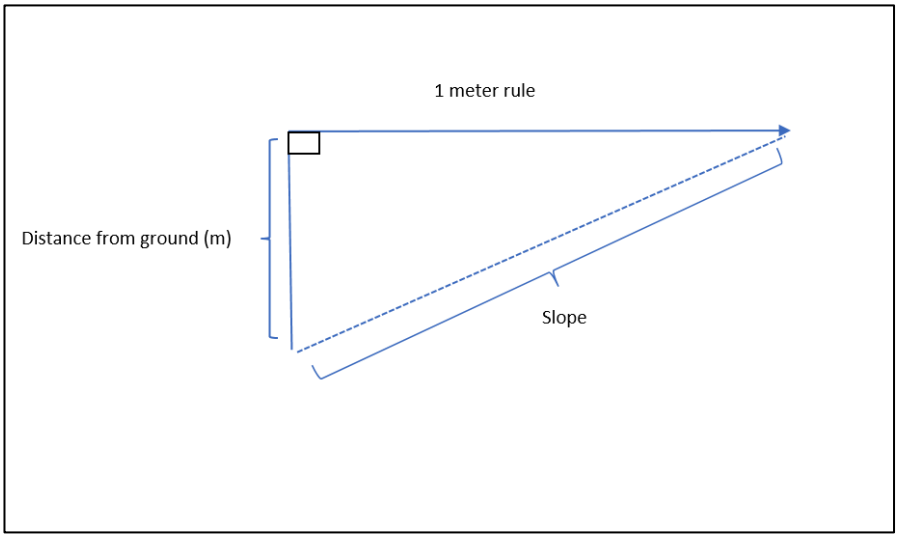

Figure 2.3: Set-up used to assess slope on specified points along transects

2.6.4 Measurement of ant functional traits

Once ants had been sorted and identified to species or morpho-species, measurements of 11 morphological traits relating to the ecological role of worker ants (Table 2.2) were taken from 5-10 individuals of each ant species (most species were represented by up to 10 individuals in the total sample) following the method of (Yates et al., 2014). Measurements were made using a camera and software installed on a Leica M125 stereo microscope.

Table 2.2: The 11 morphological traits measured on each ant and their ecological significance

Functional trait	Ecological significance
Inter-eye minimum distance	Indicative of habitat complexity (Gibb and Cunningham, 2013).
Eye width	Important for lateral view (Yates et al., 2014).
Eye length	Indicative of ants feeding guild (Weiser and Kaspari, 2006).
Head length	Indicative of ant diet (Yates et al., 2014).
Mandibular length	Indicative of foraging guild and diet.
Head width	Indicative of feeding mode (Johnson, 2000, Weiser and Kaspari, 2006).
Antennal length	Important for sensing surrounding environment (Yates et al., 2014).
Weber length (maximum)	Indicative of body size (Gotelli and Ellison, 2002), it correlates to habitat complexity (Yates et al., 2014).
Mid femur length	Indicative of speed and dispersal ability (Yates and Andrew, 2011).
Mid tibia length	Indicative of speed and dispersal ability (Yates and Andrew, 2011).
Mid tarsus length	Indicative of climbing ability (Gladun and Gorb, 2007)

Each ant species/morphospecies was initially assigned to a foraging guild (Weiser and Kaspari, 2006). (Table 2.9). However, in assessing the general occurrence of foraging guilds among the different habitats, ants were placed in either of two broad groups “generalist omnivores” or “generalist carnivores” for convenience. But for PCA analysis, the initial guilds were used.

2.6.5 Data analysis

Data were analysed using R version 3.2.4 with *lme4* and *car* packages. Models were compared using ANOVA, which was also used to eliminate factors from the model, that had the least significant effect. Akaike Information Criteria (AIC) were used to choose the best fit model (Crawley, 2002a, Symonds and Moussalli, 2011). When models were not significantly different, best fit models were selected based on having the fewest factors included. The *blmeco* package in R was used to check the best fit models for overdispersion occurring because of clustering of response variables. An observational-level random effect was included in the model to account for overdispersion (Harrison, 2014). For all statistical tests, p-values < 0.05 were considered significant.

2.6.5.1 Ant species richness, composition and relative abundance

Relative abundance was calculated for each ant species sampled across the study area, and within the different habitat types. Similarly, the Shannon Wiener diversity index was calculated for each ant species in the entire study area and within each of the three habitats, (grassland, forest edge and forest).

To assess species richness between habitats, individual-based rarefaction curves (which correct for pseudoreplication) were constructed for ant species in the overall study area and within the different habitats using the *Vegan* package in R. Rarefaction generates the statistically expected number of species in a small collection of ‘n’ individuals drawn at random from the large pool of ‘N’ individuals (Gotelli and Colwell, 2001) and rarefaction curves present the means of repeated re-sampling of all pooled individuals within a sample, plotting the average number of species represented by 1, 2,… ‘n’ individuals (Ellison et al., 2011).

The *iNext* and the *ggplot2* packages in R were used to construct interpolated and extrapolated rarefaction curves for ants within the three habitat types. Interpolation species diversity curve were based on observed abundance data. Extrapolation computes species diversity estimates and the associated 95% confidence intervals and an estimate of species diversity which may be realized by further sampling (Hsieh et al., 2016).

2.6.5.2 Variation in ant species richness among habitats

A generalised linear mixed-effect model (GLMM) with Poisson distribution was used to assess variations in ant species composition across the different habitats. The number of species was the response variable, and the independent variables were 'habitat' and, 'ant abundance' with the interactions between ant abundance and site as fixed effects. 'Transect', 'site' and 'trap ID 'were included as random effects to correct for pseudo-replication (Crawley, 2002a).

2.6.5.3 Seasonal variations in ant species composition

A GLMM with Poisson distribution was also used to check for temporal variations in ant species composition. The response variable was 'number of species' while the independent variables included 'month', 'season', 'habitat' and 'site'; with their interactions, as fixed effects. 'Transect' and 'trap ID' were included as random effects.

2.6.5.4 Ant foraging guilds

A Chi-square test was used to compare the occurrence of different foraging guilds of ants sampled in the different habitat types.

2.6.5.5 Slope across sites

One-way analysis of variance was used to assess whether slope varied significantly across the three study sites. Within-site variation was assessed using Tukey post hoc test.

2.6.5.6 Ant functional traits

The package *Factorminer* in R, was used to perform a Principal component analysis (PCA) to assess variability (assess dissimilarities and similarities) of functional traits of ants within NNFR. The PCA is used to emphasize patterns in a data set for easy

visualization and exploration. This was done based on a correlation matrix to identify traits accounting most for the variation within the ant community. The PCA was applied to values of functional traits for all ant species (ten individuals for each species, although a few species had less than five individuals), and the component loading was used to describe similarities, dissimilarities and patterns.

The PCA also was used to show the relationship between traits that contributed significantly to variations within NNFR ant community and the different factor groups (ant species, habitat, and ant foraging group) by correlating the factor groups with the different dimensions based on their coefficient estimates.

2.7 Results

2.7.1 Ant species composition abundance, and diversity

The total number of individual ants collected during this study was 2709, and they were sorted into 12 genera and 17 species/morphospecies. Thirteen species occurred in the grassland, 14 within the forest edge, and eight in the forest. The most abundant species across all habitats were *Pheidole* sp.1 and *Myrmicaria opaciventris*, with relative abundance of 43.4% and 36% respectively. Next to these but with comparatively lower relative abundance of 7.6% and 6.31% respectively, were *Lepisiota* sp. 1 and *Technomyrmex* sp. (Table 2.3).

Table 2.3: The ground-dwelling ant species sampled and their total relative abundance in the grassland, forest edge, and core forest. Asterisk denotes total relative abundance > 10 % across the three habitats. (n = number of traps in each habitat). Numbers in brackets indicate the number of traps in which respective ant species were present

Ant species	**Grassland (n =24)**	**Edge (n =42)**	**Forest (n =12)**	**Total Relative Abundance %**
Pheidole sp.1	405 (21)	479 (37)	293(12)	43.4*
Myrmicaria opaciventris	338 (20)	622 (31)	16 (2)	36.0*
Lepisiota sp. 1	137 (16)	66 (12)	3(3)	7.60
Technomyrmex sp.	147 (13)	22 (5)	2(2)	6.31
Dorylus emeryi	0 (0)	52 (7)	1(1)	1.95
Camponotus sp.1	32 (8)	8 (7)	0	1.47
Bothroponera sp.	16 (7)	10 (6)	2 (2)	1.03
Crematogaster sp.	11(1)	11(4)	0	0.81
Camponotus sp.2	7 (5)	3 (3)	0	0.37
Tetramorium sp.2	0 (0)	7 (2)	0	0.25
Pheidole megacephala	1(1)	2 (1)	1(1)	0.18
Mesoponera sp.	2 (2)	1 (1)	0	0.11
Tetramorium sp.3	1(1)	2 (1)	0	0.11
Lepisiota sp. 2	1(1)	0	0	0.04
Pristomyrmex sp.	0 (0)	1(1)	0	0.03
Tetramorium sp.1	0 (0)	0	1(1)	0.03
Tetramorium sp.4	1(1)	0	0	0.03
Total number	**1104**	**1286**	**319**	
Relative abundance	**(40.7%)**	**(47.5%)**	**(11.7%)**	

Ant species were relatively more abundant in the edge and grassland habitats than in the forest, except for *Pheidole* sp.1, which was relatively more abundant in the forest (Table 2.4).

Table 2.4: Ground-dwelling ant species in the grassland, forest edge and core forest habitat, NNFR.

Grassland	Edge	Forest
Camponotus sp.1	*Camponotus* sp.1	*Bothroponera* sp.
Camponotus sp.2	*Camponotus* sp.2	*Dorylus emeryi*
Lepisiota sp.1	*Lepisiota* sp.1	*Myrmicaria opaciventris*
Lepisiota sp.2	*Technomyrmex* sp.	*Pheidole megacephala*
Technomyrmex sp.	*Bothroponera* sp.	*Pheidole* sp.1
Bothroponera sp.	*Crematogaster* sp.	*Tetramorium* sp.1
Crematogaster sp.	*Dorylus emeryi*	*Lepisiota* sp.1
Myrmicaria opaciventris	*Myrmicaria opaciventris*	*Technomyrmex* sp.
Mesoponera sp.	*Mesoponera* sp.	*Lepisiota* sp.1
Pheidole megacephala	*Pheidole megacephala*	*Lepisiota* sp.1
Pheidole sp.1	*Pheidole* sp.1	*Technomyrmex* sp.
Tetramorium sp.2	*Pristomyrmex* sp.	*Technomyrmex* sp.
Tetramorium sp.4	*Tetramorium* sp.2	
	Tetramorium sp.3	

Shannon-Weiner diversity index calculated for ground-dwelling ant species within NNFR was 1.41. This index was 1.60, 1.23 and 0.40 for the grassland, edge, and forest habitats respectively. An individual-based rarefaction curve constructed for the total number of ants sampled across habitats reached an asymptote at about 2500 individuals (Figure 2.4), indicating that all ground-dwelling ant species present within the reserve were captured. However, similar rarefaction curves constructed for each habitat, indicate that more sampling is needed to capture all species of ants in the forest habitat because the curve for this habitat terminated at 300 individuals without reaching an asymptote (Figure 2.5). Whereas, curves for the grassland and edge habitats reached an asymptote at approximately 900 and 1100 individuals respectively.

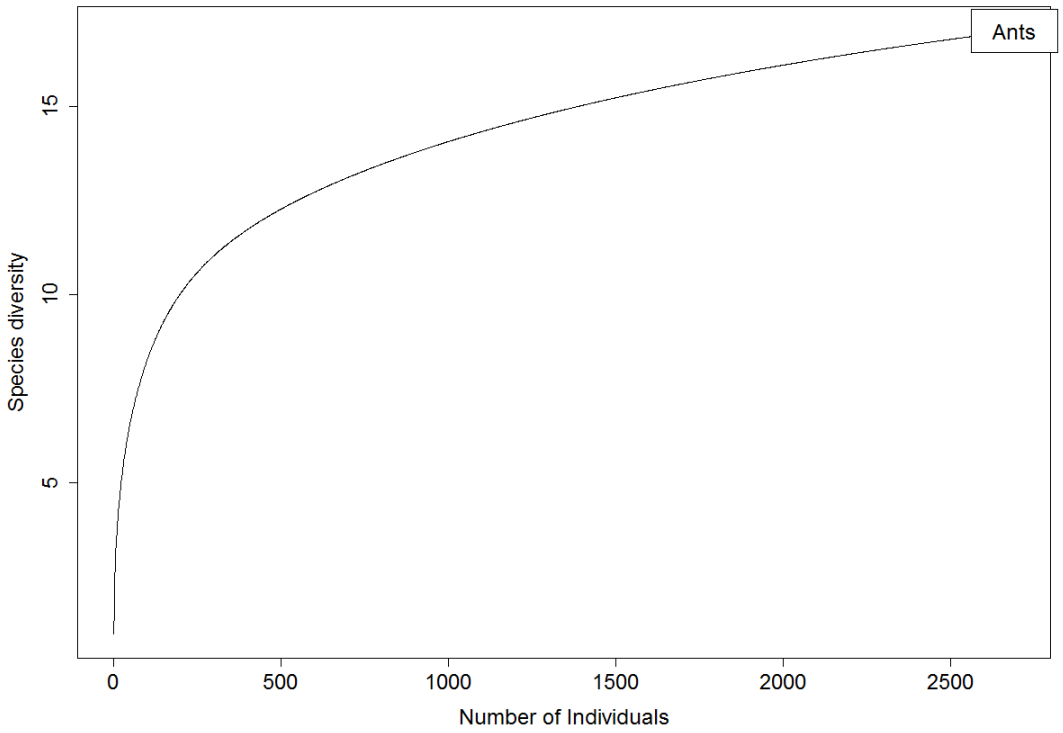

Figure 2.4: Individual-based rarefaction curve showing ant species diversity in Ngel Nyaki.

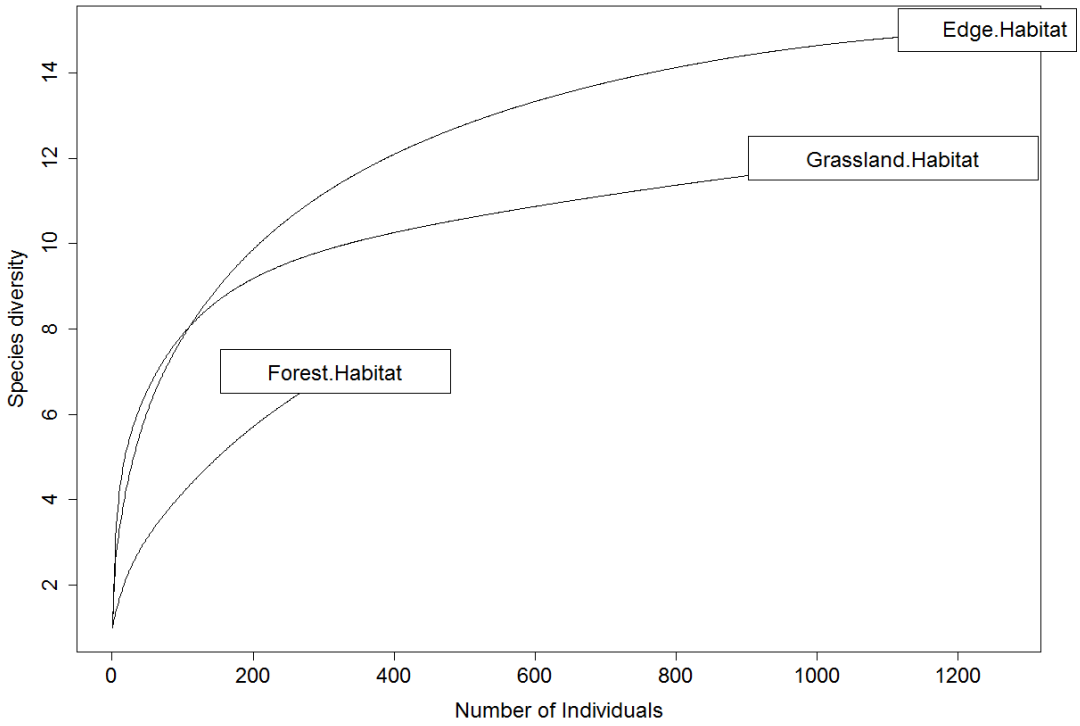

Figure 2.5: Individual-based rarefaction curve showing ant species diversity in the forest, grassland and edge habitats.

Extrapolation of the data showed that only in the forest might there be unrecorded ground-dwelling ant species (Figure 2.6).

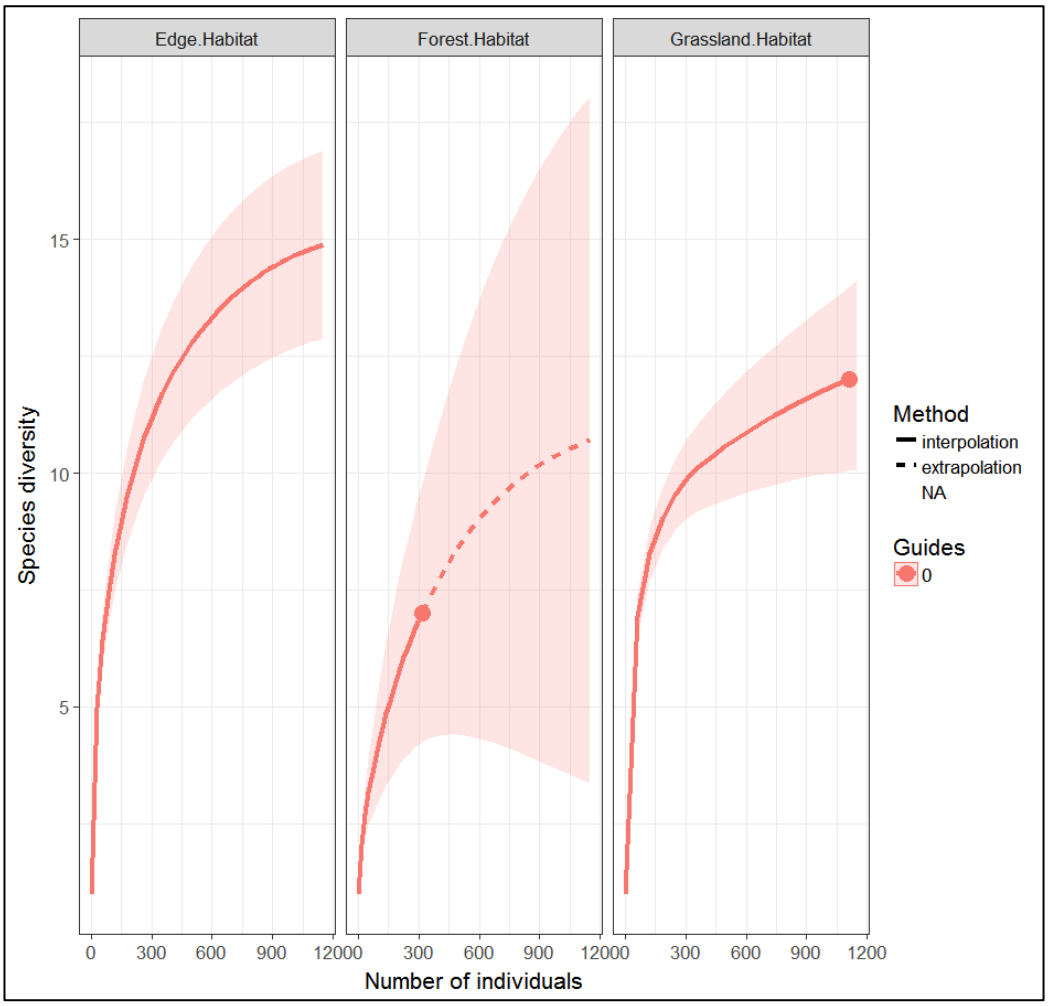

Figure 2.6: Extrapolated species diversity curves for ants in the edge, forest and grassland habitats of NNFR.

2.7.2 Spatial variation in ant species richness in Ngel Nyaki forest

Analysis of the data using a GLMM confirmed the results obtained by rarefaction (Table 2.5) and showed that species richness was positively correlated with abundance ($p < 0.0001$), while the different sites within each habitat did not affect ant species richness significantly; $p = 0.38$ (Table 2.6). However, the interaction between ant abundance and site had a significant effect on ant species richness $p < 0.0001$ (Table 2.5).

Table 2.5: Results of a generalised linear mixed model testing the effect of habitat, ant abundance, site and slope and interactions on ant species richness. Significance is denoted by asterisks at $p \leq 0.05$.

Sources of variation	Chisq	Df	p-value
Habitat	18.72	2	<0.0001*
Ant abundance	20.82	1	< 0.0001*

Table 2.6: Results of a generalised linear mixed model showing the specific effect of habitat, ant abundance, site and interactions on ant species richness. Significance is denoted by asterisks at $p < 0.05$

	Ant species Richness		
	Estimates	***Z-value***	***p-value***
Fixed Parts			
(Intercept)	0.21	3.20	<.01*
Forest	-2.3	- 1.60	0.10
Grassland	0.34	3.42	<.001**
Number of ants	0.01	4.56	<.0001***

2.7.3 Temporal variation in ant species richness

The main sources of temporal variation in ant species richness, were minimum temperature and the interaction between year and season ($p < 0.05$) whereas, neither year nor season on their own had a significant effect on ant species richness ($p = 0.63$ and 0.07 respectively) (Table 2.7). Year 2016 and wet season had a significant positive effect on ant species richness ($\beta = 0.49$, p-value <0.05) (Table 2.8), but richness was not affected significantly by the interaction between season and minimum temperature ($\beta = 2.30$, p-value =0.14) (Table 2.7).

Table 2.7: Generalized linear mixed model with Poisson distribution showing the effect of year, season, site, minimum temperature and their interactions on ant species richness. Significance is denoted by an asterisk at $p < 0.05$

Sources of variation	**Chisq**	**df**	**p-value**
Year	0.23	1	0.63
Season	3.14	1	0.07
Minimum Temperature (ºC)	9.13	1	<0.001*
Year: Season	6.10	1	<0.05*
Season: Minimum Temperature (ºC)	2.30	1	0.13

Table 2.8: Generalized linear mixed model showing the specific effect of year, season, minimum temperature and their interactions on ant species richness. Significance is denoted by an asterisk at $p < 0.05$

	Ant species Richness		
	Estimates	***Z-value***	***p-value***
Fixed Parts			
(Intercept)	-0.28	-0.63	0.52
Year 2016	-0.36	-2.27	<0.05*
Wet season	-2.05	-1.99	<0.05*
Minimum Temperature (ºC)	0.06	1.84	0.06·
Year 2016: Wet season	0.49	2.47	<0.05*
Wet season: Minimum Temperature (ºC)	0.10	1.51	0.12

2.7.4 Variation in slope across sites

Slope varied significantly between the three different study sites $F_{2,361} = 35.6$, $p < 0.0001$.

2.7.5 Foraging guilds of ant species

Ant genera and species were assigned to foraging guilds according to the literature (Table 2.9), but these were reduced to two broad categories namely: generalist omnivores and generalist carnivores. Eighty-six percent (86%) of the ants were categorised as generalist omnivores, and 14 % as generalist predators/carnivores.

Table 2.9: Foraging guilds of ant species sampled at Ngel Nyaki Forest Reserve, Nigeria.

Ant species	Foraging Guild
Camponotus sp.1	Omnivorous (Feldhaar et al., 2007)
Camponotus sp.2	Omnivorous (Feldhaar et al., 2007)
Lepisiota sp.1	Generalists (Brown, 1973)
Lepisiota sp. 2	Generalist (Brown, 1973)
Technomyrmex sp.	Carnivorous (Bolton, 2007)
Bothroponera sp.	Predators (Bolton, 2003)
Crematogaster sp.	Predators (Richard et al., 2001)
Dorylus emeryi	Predators (Hölldobler and Wilson, 1990)
Myrmicaria opaciventris	Generalist omnivores (Kenne and Dejean, 1999)
Mesoponera sp.	Predators (Agbogba ,1985)
Pheidole megacephala	Generalists (Hoffmann et al., 1999)
Pheidole sp.1	Omnivorous (Byrne, 1994)
Pristomyrmex sp.	Carnivorous (Wang, 2003)
Tetramorium sp.1	Predators (Garcia and Fisher, 2014)
Tetramorium sp.2	Predators (Garcia and Fisher, 2014)
Tetramorium sp.3	Predators (Garcia and Fisher, 2014)
Tetramorium sp.4	Predators (Garcia and Fisher, 2014)

Occurrence of the two broad categories of foraging guild (generalist omnivores and generalist carnivores) differed significantly among the three habitat types; grassland, edge and forest habitat (Generalist omnivores: $\chi^2 = 121.94$, df = 2, p-value < 0.0001; Generalist carnivores: $\chi^2 = 23.1$, df = 2, $p < 0.0001$,). Relative frequency of generalist omnivores and generalist carnivores were higher in the grassland and the edge habitat compared to the forest habitat (Figure 2.7).

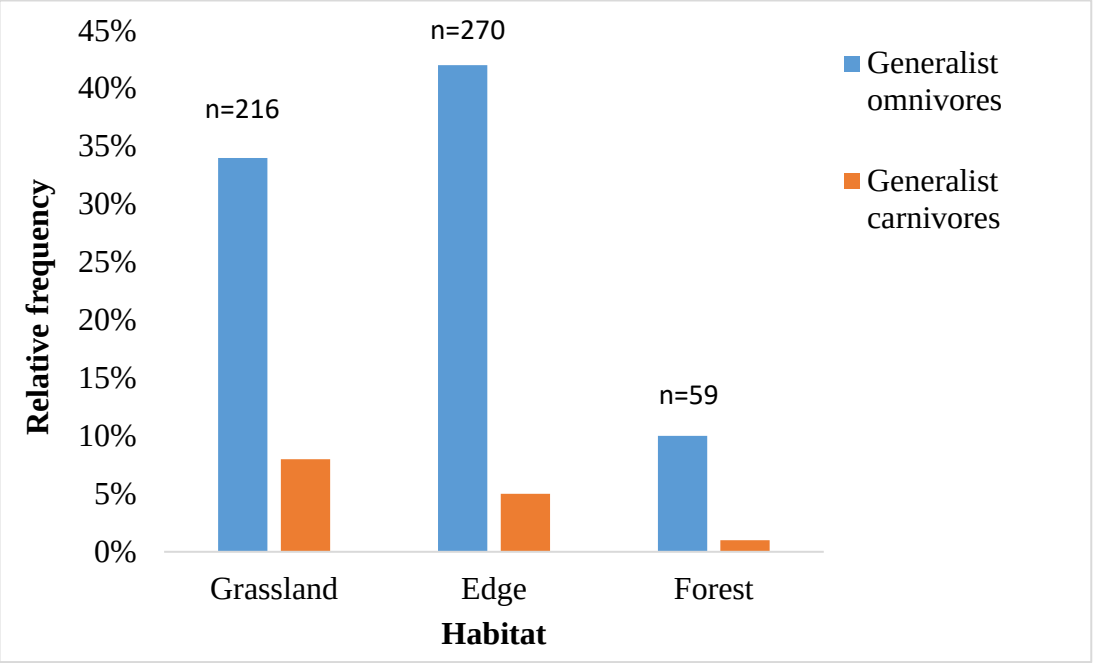

Figure 2.7: Foraging guilds of ants sampled within Ngel Nyaki Forest reserve across the edge, forest and grassland habitats. The proportions on the vertical axis were derived by dividing the frequency of occurrence of guilds by the total number of trap collections in which ants were present (n = 216 grassland; edge 270; forest 59)

2.7.6 Ant functional traits

One hundred and seven individual ants belonging to 17 species/morpho-species were measured and analysed using a PCA. The first and second dimensions of the PCA explained 83.7% of the variations in functional traits of ant species within NNFR Dimension 1 explained 73.9% and dimension 2 accounted for 9.8% of the variance (Table 2.10).

Six functional traits accounted for the variation in the first two dimensions of the PCA. These were: mid-tibia length, mid-femur length, antennal length, weber length and mid-tarsus length. Mandibular length was one of the most important traits accounting for variation within the ant community, and it had the longest variable vector compared to the other variables. All other variables had similar vector lengths apart from eye width and inter-eye distance which had shorter variable vectors (Figure 2.10).

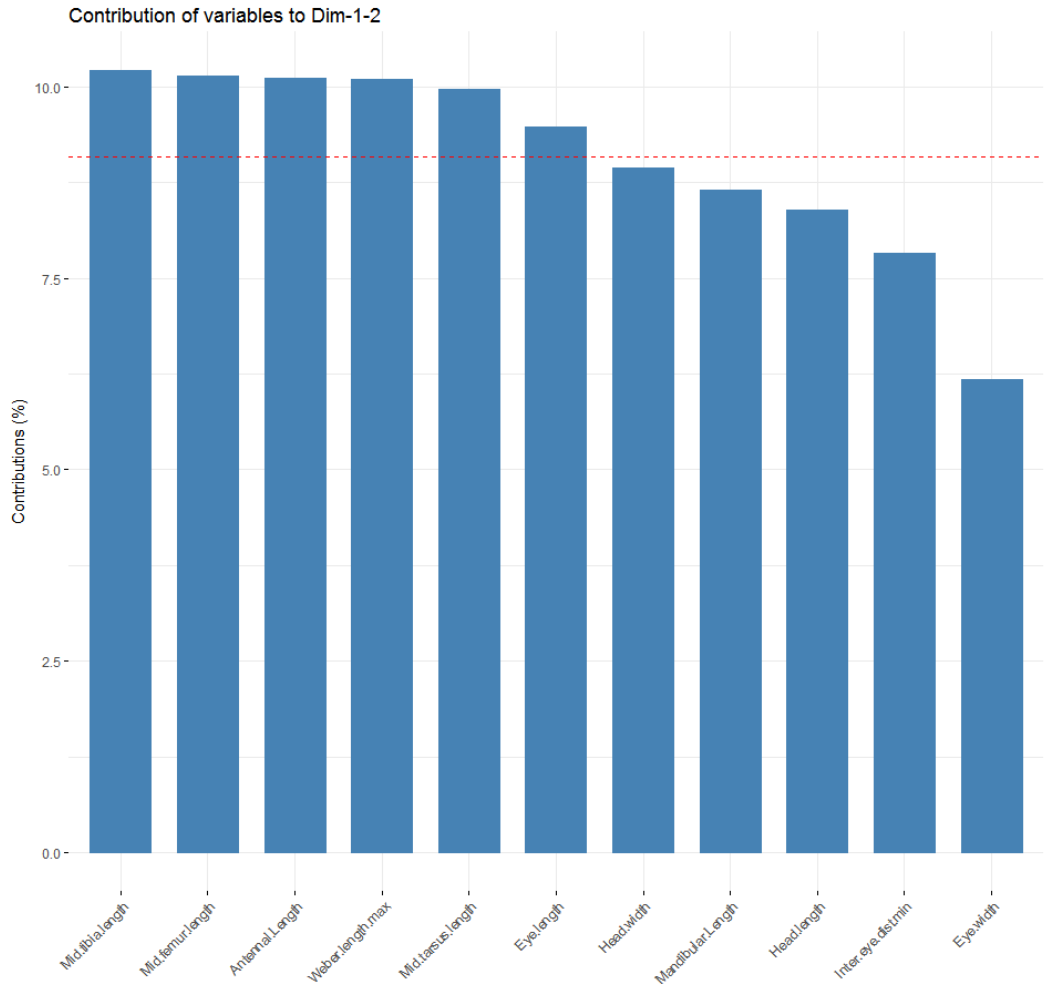

Figure 2.8: Representation of the contribution of each functional trait to the first two dimensions of the PCA. Functional traits with values above the red line are those that contributed the most to variations in the PCA.

2.7.7 Description of ant functional trait PCA

The first dimension of variability accounted for 73.9% of the variance with component coefficients (correlation coefficients between variables and factors) ranging from 0.68 - 0.95. It was strongly correlated to six of the seven contributing variables; weber length, mid tibia length, antennal length, mid tarsus length, mid femur length and eye length. All, except eye length, had high component coefficient values ≥ 0.95 and accounted significantly for variations in the first dimension (Table 2.10). The second dimension of variability correlated weakly; with low component

coefficients that ranged from -0.21- 0.72. However, mandibular length and inter-eye distance accounted significantly for variations in the second dimension with significant coefficients of 0.72 and 0.51 respectively (Table 2.10).

Table 2.10: Summary of Principal Component Analysis performed on ant functional traits showing the component coefficient of each trait and variance explained by each dimension. Asterisks denote significant component coefficient.

Variable	Dimension 1	Dimension 2
Inter-eye minimum distance	0.68	0.51*
Eye width	0.69	-0.30
Eye length	0.91*	-0.21
Head length	0.87	-0.12
Mandibular length	0.52	0.72*
Head width	0.85	0.29
Antennal length	0.95*	-0.12
Weber length (maximum)	0.96*	0.00
Mid femur length	0.95*	-0.16
Mid tibia length	0.95*	-0.14
Mid tarsus length	0.95*	-0.15
Percentage variance	73.9%	9.8%

The significant component coefficients in the first dimension of the PCA (Table 2.9) are associated with traits related to body size (weber length, mid femur length, and mid tarsus length). Ants (*Camponotus sp*. 2, *Camponotus sp*. 1, *Mrymicaria*

opaciventris, Bothroponera sp) with positive coefficients in this dimension, had larger bodies, longer legs and longer eye length. The reverse was the case for ants (*Dorylus emeryi, Lepisiota* sp.1, and *Technomyrmex*) that had negative coefficients (Table 2.10).

The second dimension was associated with mandibular length and inter-eye distance, so that the species *Bothroponera* sp., *Mesoponera* sp. and *Mrymicaria opaciventris* with positive component coefficients had markedly longer inter-eye distance and longer mandibles than than *Lepisiota* sp.1, *Camponotus sp*. 1, and *Camponotus sp*. 2 with negative coefficients (Table 2.10).

Table 2.11: The contributions of each ant species to variations in functional trait in the first two dimensions of the PCA with their coefficients estimates.

Dimension	Ant species	(R^2)	Coefficient Estimates
Dim 1		$R^2 = 0.90$	
	Camponotus sp. 2		6.40
	Camponotus sp. 1		3.77
	Bothropenora sp.		2.59
	Myrmicaria opaciventris		2.50
	Dorylus emeryi		-2.20
	Lepisiota sp.1		-1.96
	Technomyrmex sp.		-2.2
Dim 2		$R^2 = 0.77$	
	Bothroponera sp.		1.92
	Pheidole megacephala		1.71
	Mesoponera sp.		1.93
	Lepisiota sp. 1		-0.84
	Camponotus sp. 1		-1.18
	Camponotus sp. 2		-1.41

Ant species associated with both grassland and forest edge tended to have a larger body size (positive component coefficient, 2.86), while ants associated with forest edge and forest habitat, as well as those confined to edge habitats tended to have smaller body size (Table 2.12).

Table 2.12: Contribution of macrohabitat to variations in functional trait Principal Component Analysis; Dimensions 1 and 2 and their PCA coefficients.

Dimension	Habitat characteristics	*(R^2)*	Estimates
Dim 1		R^2= 0.22	
	Grassland-edge		2.86
	Edge		-0.88
	Edge-forest		-1.53

The ant foraging guild with a positive coefficient (omnivorous) on the first dimension of the PCA had larger body size, while ants categorised as generalists, carnivorous and predators, had small body size. The second dimension of the PCA shows that ants that are predators had longer mandibular length while omnivorous ants on the second dimension had shorter mandibular lengths (2.13).

Table 2.13: Contribution of ant foraging guild to variations in functional trait Principal Component Analysis; Dimension 1, and 2 and their PCA coefficients.

Dimension	Feeding guild	*(R^2)*	Estimates
Dim 1		R^2= 0.25	
	Omnivorous		3.19
	Generalists		-1.03
	Carnivorous		-1.93
	Predators		-0.22
Dim 2		R^2= 0.19	
	Predators		0.61
	Omnivorous		-0.39

2.7.8 Functional trait similarities and dissimilarities among ant species

Figure 2.9 illustrates the clustering of ant species on the PCA. Three ant species: *Camponotus* sp.2, *Camponotus* sp.1, and *Myrmicaria opactventris* stood out as having similar functional traits. They occupied the largest cluster in the individual PCA plot. Other ants (*Pheidole* sp.1, *Pheidole megacephala*, *Lepisiota* sp.1, *Lepisiota* sp.2 *Crematogaster* sp, *Dorylus emeryi*, *Technomyrmex* sp., *Tetramrium* sp. 1, *Tetramrium* sp. 2, *Tetramrium* sp. 3, *Tetramrium* sp. 1 *Mesoponera* sp., *Bothroponera*) occupied several smaller overlapping clusters which indicates similarity with slight differences in functional traits among the different ant species (Figure 2.9).

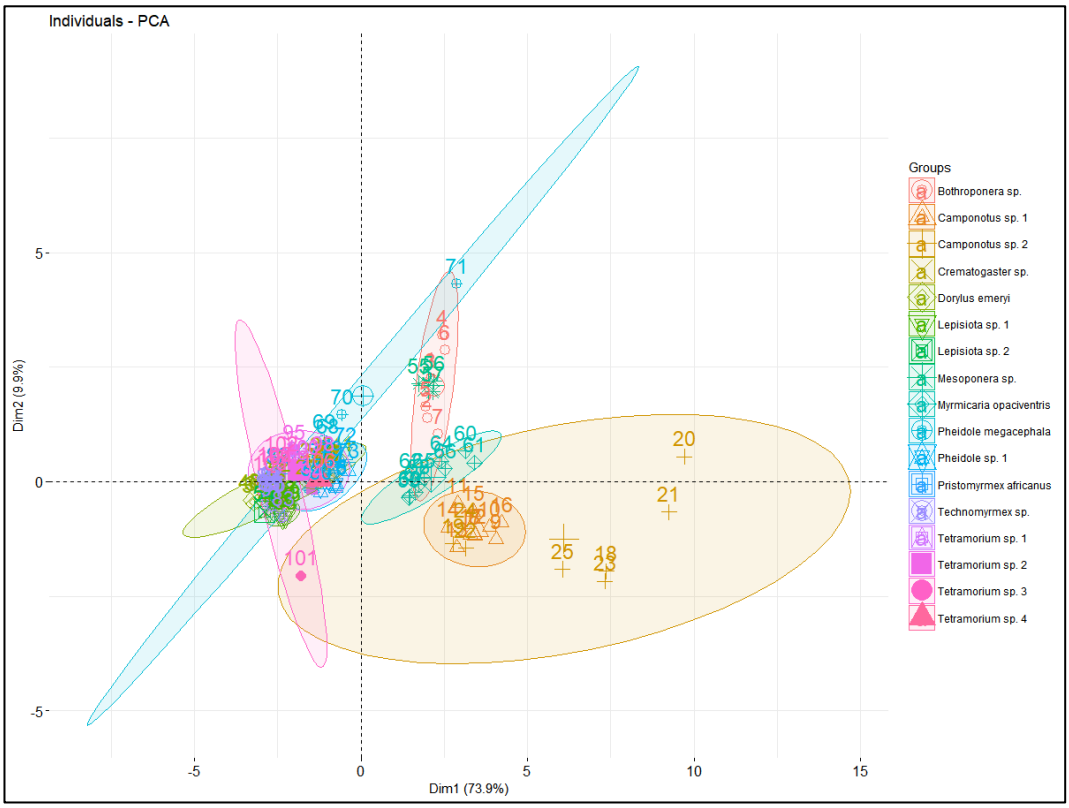

Figure 2.9: Representation of individual ant species along Dimension 1 and 2 of the Principal Component Analysis of ant functional traits. Numbers refer to individual ants and symbols to the different species. The ellipse shows groups with similar traits.

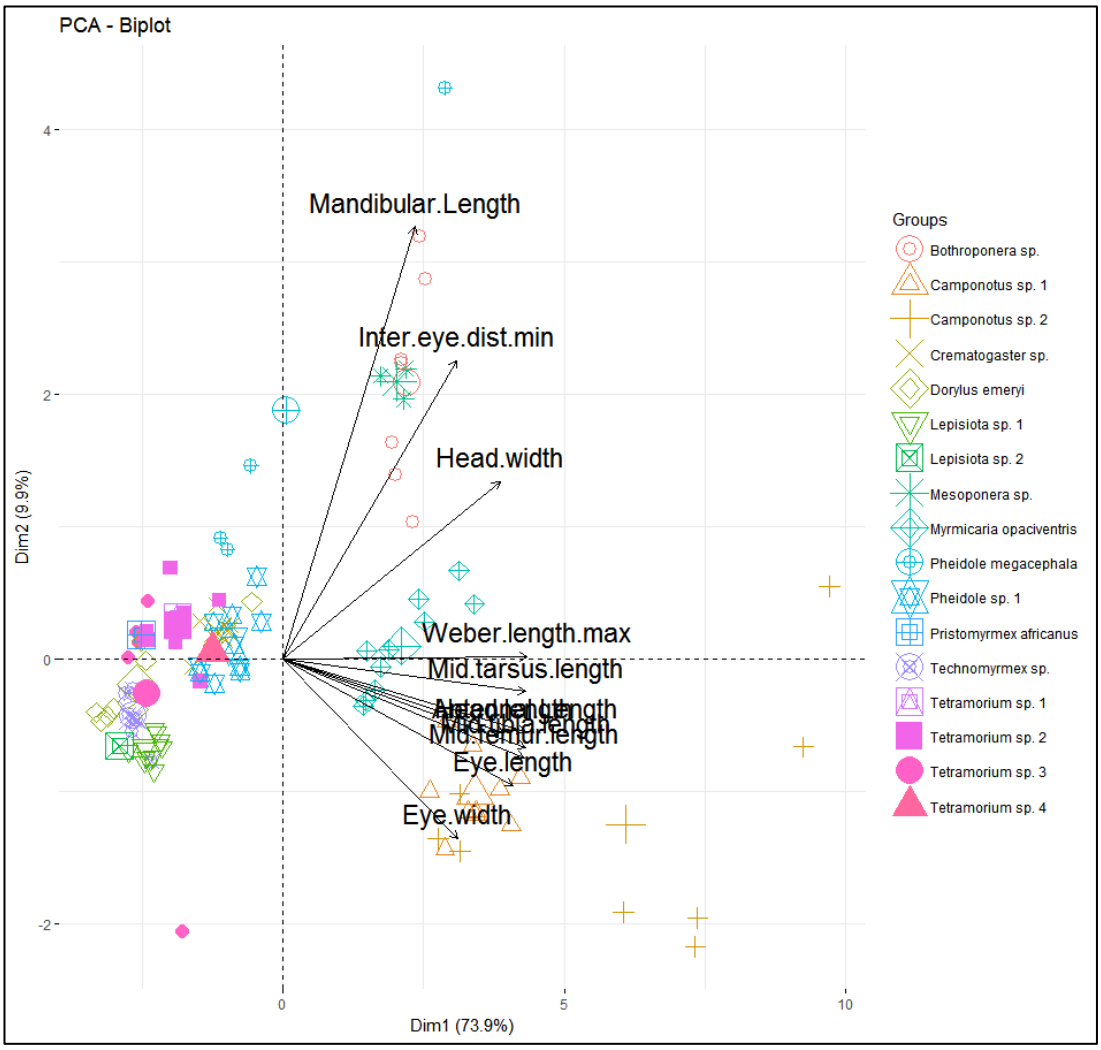

Figure 2.70: Biplot of functional traits and ant species along dimension 1 and 2.

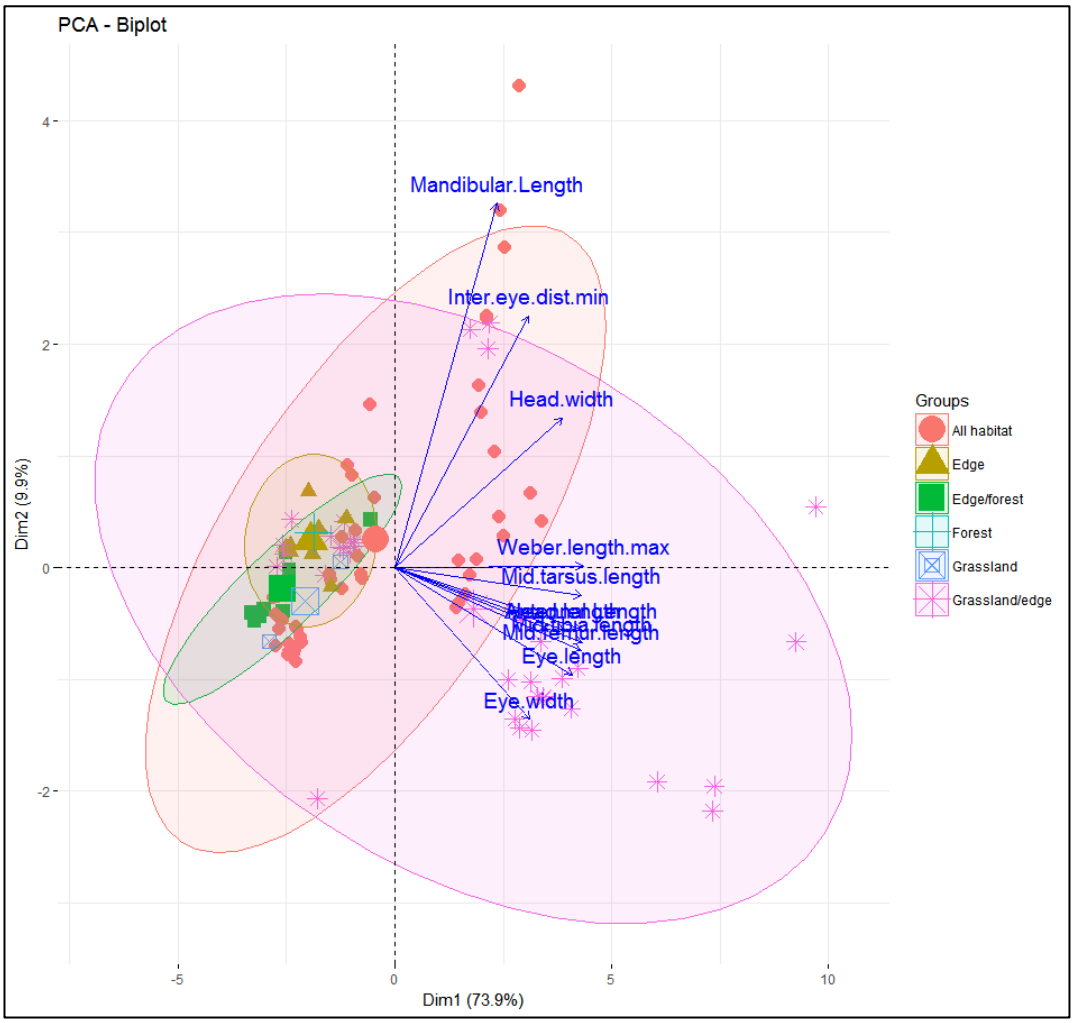

Figure 2.81: Biplot of functional traits and ant species along dimension 1 and 2. The eclipse shows group with similar trait values. Majority of the ants found in grassland and forest edge (purple asterisks) are distinct from the rest.

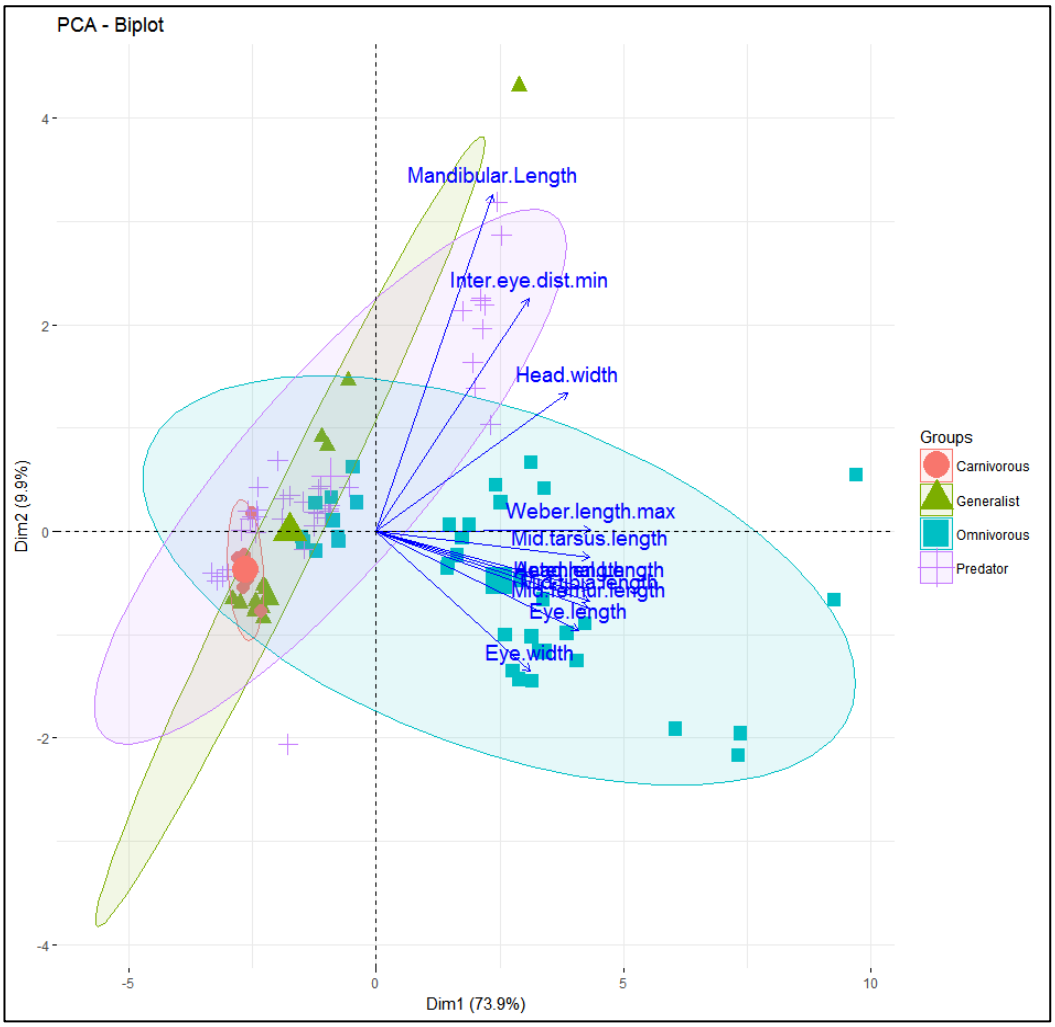

Figure 2.12: Biplot of functional traits and ant species along dimensions 1 and 2. The eclipse shows groups with similar trait values? (specialised guilds appear more constrained in the trait ordination space).

2.8 Discussion

2.8.1 Ant species richness and diversity

This study is the first to assess species richness of ground dwelling ants in a West African montane landscape comprising grassland, forest edge and forest habitats. Over 12 months of trapping, I recorded 17 ant species, and the diversity index calculated for ants across all three habitats was 1.41. This is low ant diversity in comparison to both lowland tropical forests (Longino et al., 2002) and a montane forest habitat in Kenya (Fischer, 2012).

Tropical ecosystems are known to have high ant species richness and abundance (Hölldobler and Wilson, 1990, Leal et al., 2012), it is typical to record about 100 ant species in one hectare in tropical lowland forests (Arnan et al., 2011). However, it has been widely demonstrated that species richness declines with increasing elevation (Beattie et al., 2002, Brown, 1973, Olson, 1994, Stevens, 1992), although a peak in ant species richness has been reported to occur at mid-elevations (Bishop et al., 2014). Studies of ant species composition in most other montane ecosystems show a decline in species richness with altitude (Table 2.1) (Brühl et al., 1999, Dunn et al., 2009, Sabu et al., 2011). However, as referenced above, a study in a montane forest in Kenya (Kakamega forest) recorded 329 ant species. This is the highest number of ant species ever recorded in a montane landscape at an altitude above 1500 m. In contrast to my study, in the Kakamega study the forest had higher ant species richness than the grassland habitat (Fischer, 2012). To explain this finding the authors suggested that Kakamega forest is a remnant of a once continuous, highly diverse Equatorial-African rainforest system, and might explain the high ant species richness recorded in the area relative to other montane landscapes (Fischer, 2012, Wagner et al., 2008). Corroborating my study, a study in a South African montane forest recorded low ant species richness of about 28 species (Kotze and Samways, 2001), which is still higher than NNFR. The low ant species richness in NNFR, like most other montane forest regions may be driven by the unique climatic conditions such as the low ambient temperature of montane regions. It might also be due to its prolonged isolation as one of the few remaining protected and forest fragments on the Mambilla plateau.

2.8.2 Effect of habitat on ant species richness and relative abundance.

Ant species composition and richness differed significantly across the different habitat types (Table 2.4). The edge habitat was the most species-rich, followed by the grassland habitat. The forest habitat had the least number of species (Table 2.5). However, ant species richness and diversity decreased from grassland to forest. A study carried out in a montane forest in South Africa similarly found ant species richness to shift with variation in habitat (Kotze and Samways, 2001). They found a decrease in ant species richness along the grassland to forest gradient with fewer

species in the forest and an increase in the edge and grassland habitat (Kotze and Samways, 1999). This suggests that the grassland habitat might be important for maintaining ant species richness and diversity within Afromontane landscapes. Typical Afromontane landscapes consist of a forest fragment, and a distinct edge that abuts grassland. The origin of these grasslands is debatable. But some suggest it could be is an artefact of previous anthropogenic disturbance (e.g., grazing, fire, and logging) in what was once a uniform forested landscape (Everard, 1986, Geldenhuys, 1989, Kapos, 1997). Recent conservation efforts within Afromontane landscapes aims to protect remnant forest fragments from degradation (Roselli, 2014, Wubet et al., 2003) and possibly restore degraded adjoining grassland. Grassland habitats in Afromontane landscapes are generally perceived as less valuable than forest in terms of the ecosystem services they provide (De Foresta and Michon, 1996, Kotze and Samways, 2001). However findings of this study and the study by Kotze and Sammy (2001) in South Africa suggest that protecting Afromontane grasslands from anthropogenic activities alongside forest fragments is important for maintaining the diversity of ant community. The higher diversity in the grassland habitat relative to the forest habitat suggests that not preserving grasslands could lead to a decrease in ant diversity in the whole landscape (Kotze and Samways, 1999, Kotze and Samways, 2001).

The edge habitat had a higher number of species (14 species with a mean of 15 individuals per trap) than the grassland (13 species with a mean of 23 individuals per trap). However, the grassland had the higher diversity index of 1.60, relative to 1.23 in the edge. Edges habitats are usually structurally more heterogeneous (Gignac and Dale, 2005) and as such, invertebrates have more niches to occupy within the edges (Kotze and Samways, 2001, Warfe et al., 2008). This might explain why more species were recorded in the edge than the grassland and forest habitat. However, the grassland was more diverse than the edge habitat. The higher diversity score in the grassland indicates a more even distribution of ant species in the grassland than in the edge habitat. This is consistent with a study in a South African montane forest that observed no biological edge effect on ant species richness, with the grassland habitat having higher species diversity than edge and forest (Kotze and Samways, 2001). In this study, ants that occurred in both the grassland and edge habitats had a

wide range of functional traits; occupying the largest portion of the PCA orthogonal space (Figure 2.12). These habitats (grassland and edge) harboured most of the ant species that occur in NNFR and as such, it is expected that ants that occur in both the grassland an edge habitat, will be capable of diverse functional roles due to their variability in functional traits. This emphasizes the importance of the grassland along with the edge habitat in maintaining ant species richness in this Afromontane landscape.

The forest habitat was the least diverse in both ant species diversity (0.4) and relative abundance (8 species with a mean of 13 individuals per trap) compared with the grassland and the edge habitats. Of the eight ant species found in the forest habitat, three had less than four individuals recorded, and three others were represented by only one individual. This indicates that apart from *Pheidole* sp.1 which was abundant, the ground-dwelling ant assemblage in the forest consists mainly of rare species that may require long-term sampling to be captured. This may explain why the individual-based rarefaction curve for the forest habitat did not reach an asymptote (Figure 2.4). Aggressive and competitive behaviour between *Pheidole* sp.1 and other ant species was not observed in the forest habitat during experiments and focal observation, suggesting that the highly abundant *Pheidole* sp.1 is probably not a predator of other ant species. However, the low abundance of other ant species in the forest habitat might be due to cooler temperatures in the forest habitat due to lower sun radiation reaching the forest floor compared to the edge and the grassland habitats. Forest habitats are usually cooler than edge and grassland habitats (Magura et al., 2001). However, the occurrence of the omnivorous generalist ant *Pheidole* sp.1 in high abundance in the forest habitat suggests that it is adapted to different conditions being a more generalist species. This may also explain its ubiquity in NNFR.

2.8.3 Temporal variation in ant species richness

Overall, season had no significant effect on ant species richness (p-value =0.07, Table 2.6), although a decrease in ant species richness was observed during the wet season whereas the dry season did not affect ant species richness (Table 2.7). Other studies found a positive correlation between ant species richness and rainfall (wet

season) (Davidson, 1977, Kaspari and Weiser, 2000), this is, however, contrary to findings in Australia and South America where the reverse was the case (Medel, 1995, Morton and Davidson, 1988). Year 2015 had no significant effect on ant species richness, but the interaction between year and season had a significant effect on ant species richness. The wet season of the year 2016 had a significant positive effect on ant species richness with three more species recorded in wet season than in the dry season.

Minimum temperature had a positive effect on ant species richness in NNFR. The effect of temperature on species richness varies across different geographic regions (Hawkins et al., 2007). Plants and animal species in tropical montane habitats are usually adapted to cool temperatures and humid conditions (Wikramanayake, 2002). This might explain why minimum temperature had a positive effect on ant species richness in NNFR (Table 2.8). Small ants are usually prone to desiccation risk from high temperatures and low humidity (Kaspari, 1993), as such temperature affects their behaviour which indirectly affects species richness (Kaspari and Weiser, 2000). Lower temperatures and humid conditions (from high rainfall) are likely to lower physiological stresses like desiccation (Kaspari, 1993, Sanders et al., 2003) which ants are prone to (Kaspari, 1993). That I found ant species richness to correlate positively with minimum temperatures could be an indication that ants in NNFR thrive in low temperature conditions. Higher temperatures poses a desiccation risk to small ants (Kaspari, 1993). Fourteen out of the 17 ant species sampled in NNFR are small ants with body size < 0.2 mm. An adaptation to small body size in regions such as NNFR with low temperature and high humidity makes sense because there is lower risk of desiccation because these unique montane forest climatic conditions.

2.8.4 Dominant ant species in Ngel Nyaki forest reserve

Two ant species; *Pheidole* sp. 1 and *Myrmicaria opaciventris* were the numerically dominant ant species in NNFR with relative frequency of 43.4% and 36 % respectively and may be the ant species with the most impact on ecological processes within the area. The behaviour of these species woulde determine the nature of their interactions with other species as well as the environment.

2.8.4.1 *Pheidole* sp. 1

From this study, *Pheidole* sp.1 was the most abundant species. It was sampled at relatively high densities in all of the three habitats and study sites. This is not surprising, as ants from this genus are found to be the most abundant in most tropical ecosystems (Wilson, 2003). The genus *Pheidole* is a morphologically diverse genus with a worldwide distribution (Wilson, 2003, Zara and Fowler, 2005) containing over 900 described species that vary mainly in body size (Pie and Traniello, 2007). Despite the high species richness of the genus *Pheidole*, it has the highest proportion of undescribed and unidentified morphospecies in Africa (Fischer, 2012, Hita Garcia et al., 2009). Afrotropical *Pheidole* fauna is lacking in identification keys (Fischer, 2012) as such it is presently difficult to identify some morphospecies to species level especially in places where baseline data on ant species composition is lacking. The *Pheidole* sp.1 sampled in this study could not be described to species level. Afrotropical *Pheidole* fauna is lacking in identification keys (Fischer, 2012) as such it is presently difficult to identify some morphospecies to species level especially in places where baseline data on ant species composition is lacking. Whether *Pheidole* sp.1 sampled in NNFR is confined to Afromontane landscape alone is undeterminable at this time. It could be inferred from the abundance of *Pheidole* sp.1 in all the three habitats sampled in NNFR, that it has a significant impact in the area through its interactions. Hence it is important to understand its interactions with plants, animals and the environment to determine the ecological role it plays in NNFR. Because it is a generalist omnivore, it can be expected to interact with, and affect the arthropod and plant communities. Other studies have shown that ants from the genus *Pheidole* affect arthropod community, prey on seeds and also act as important seed dispersers (Byrne, 1994, Kaspari, 1996, Levey and Byrne, 1993), although most species are small and may not be able to move large seeds (Kaspari and Weiser, 1999).

2.8.4.2 Myrmicaria opaciventris

Myrmicaria opaciventris was next to *Pheidole* in numerical dominance. However, it was abundant mainly in the edge and grassland habitats. Only 16 individuals were sampled from within the forest habitat. This is consistent with a study carried out in an East African rainforest. In that study, *M. opaciventris* was the most abundant

species sampled, but it was confined to open habitats and did not occur in closed habitats (Fischer, 2012). This indicates that it is an open habitat specialist that occurs mainly in grasslands and edge habitats. It has also been found to occur in altered landscapes such as agricultural landscapes where it preys on other insects. On that account, it has been considered for use as a biological control agent for insect pests (Kenne and Dejean, 1999, Kenne and Dejean, 1997).

2.8.4.3 Pheidole megacephala

This study is the first to report the occurrence of *Pheidole megacephala* in South-eastern Nigeria and specifically, in an Afromontane forest. Reports of its occurrence in Nigeria remain sketchy with speculation that it occurs in northern and western Nigeria (Sudd, 1962, Wetterer, 2012). This species is an important and aggressive invasive ant species that has been observed to affect the population of ground-dwelling arthropods in different parts of the world (Heterick, 1997, Wetterer, 2012) and native ant communities in Australia (Hoffmann et al., 1999, Young, 2000) and Hawaii (Jones et al., 2001). It was recorded in low density at NNFR with a total relative abundance of 0.18.%. However, in many areas where it is found, it occurs at high densities (Jones et al., 2001, Majer et al., 2000, Wetterer, 2012), constituting about 99.6% of ants collected in infested sites in Hawaii (Jones et al., 2001) and Australia (Majer et al., 2000). The rarity of this species in NNFR may be due to environmental constraints of high altitude and low temperature, which might limit its invasiveness and overall impact on ant and overall arthropod community in this area. Its rarity may also indicate that it is not invasive in this environment.

2.8.5 Variations in ant feeding guilds

Ants in the generalist omnivore guild were the most abundant recorded in NNFR; they occurred in 86% of the trap collections. Their proportion differed significantly across the three habitat types ($\chi^2 = 121.9$, df = 2, p-value < 0.0001). The high proportion of omnivorous generalist ants in NNFR suggests that they impact on ecosystem processes within NNFR; especially those species that occurred in the edge and grassland habitat where they had the highest abundance and species richness. The majority of ants are omnivores or generalists (Hölldobler and Wilson, 1990), as our finding that omnivorous ants were the most common in NNFR is consistent with

this. Their diet is usually made up of plant material such as nectar, and insect prey (Hölldobler and Wilson, 1990). They play an important role in ecosystems by their ability to influence the population of other ground dwelling arthropods (as predators) and also affect plant community through interactions such as seed predation (Carroll and Risch, 1984) and seed dispersal (Corlett, 2009, Cuautle et al., 2005, Oquias, 1994). The fact that ants belonging to the generalist omnivore group are responsible for most ant-plant interactions (Hölldobler and Wilson, 1990) indicates their importance in important interactions such as seed dispersal. Ant-seed dispersal has been shown to aid plant recruitment and regeneration in other tropical ecosystems (Christianini and Oliveira, 2009, Christianini and Oliveira, 2010, Gallegos et al., 2014).

Ants in the generalist carnivore group accounted for the lowest proportion of the ants sampled within NNFR and occurred in only 14% of the total traps collections (Figure 2.5). Despite occurring in low proportions, one important predatory ant species *Dorylus emeryi* was sampled within NNFR. It occurred mainly in the edge habitat (Table 2.2). This species belongs to a genus popularly known as the army ants. It is an obligate predator that raids in swarms and retrieves invertebrate prey to its nest (Brady, 2003, Gotwald Jr, 1978, Hölldobler and Wilson, 1990). Due to this behaviour, it significantly impacts arthropod and invertebrate communities in tropical ecosystems (Gotwald Jr, 1995, Kronauer et al., 2007). The high ant species richness and the high proportion of ants sampled in the edge habitat might explain why *Dorylus emeryi* occurred mainly in the edge habitat. The high species richness of ants in the edge habitat may reflect a high composition of ground dwelling invertebrates in the edge habitat. *Dorylus emeryi* occurred at a low density in NNFR with an overall relative density of 1.95 (Table 2.2). This might be because of the unique abiotic conditions such as altitude and low temperature in Afromontane landscapes which is likely to limit their abundance, occurrence, and their effect on the arthropod and invertebrate community within the reserve.

2.8.6 Ant functional traits across habitats

From this study, morphological (functional) trait differences within the ant community in NNFR, were apparent at the macrohabitat scale (Figure 2.10) although

macrohabitat explained only about 22% of the variance in ant functional trait in the first dimension of the PCA (Table 2.10)

Ants that occurred in simpler open habitat such as the grassland and parts of the edge habitat, had larger body sizes than ants which occurred in the closed and more complex edge and forest habitats. This is consistent with size-grain hypothesis confirmed in several studies which show that ants in structurally simpler habitats have larger bodies and longer legs than those in more heterogeneous habitats (Gibb and Cunningham, 2013, Kaspari, 1993, Yates and Andrew, 2011, Yates et al., 2014). Ants that occurred in closed and more complex edge and forest habitats had smaller body sizes and shorter legs than those in the open and structurally simpler grassland habitats. The edge and forest habitat are usually more complex and are characterised by dense twigs, and leaf litter in which smaller ants with shorter legs can easily infiltrate interstices that large-bodied ants with longer legs cannot (Farji-Brener et al., 2004, Kaspari and Weiser, 1999).

The key traits responsible for variations in NNFR ant community were weber length and leg length (mid femur, mid tibia and mid tarsus length) (Table 2.7). Weber length is indicative of ant body size (Gotelli and Ellison, 2002), and is one of the most important morphological traits that can constrain where ants can forage (Gibb and Parr, 2010) while leg length is indicative of speed and dispersal ability (Yates and Andrew, 2011). It is expected that ants in open grassland of NNFR would forage further distances- hence the larger body size and longer legs. Smaller invertebrates including ants are prone to desiccation stress, as such, habitats that are open with lower moisture content selects for ants with larger body sizes that can avoid desiccation more efficiently than smaller bodied invertebrates (Kaspari, 1993, Kaspari and Weiser, 2000).

2.8.7 Ant functional traits and feeding guilds.

In recent years, understanding how functional traits relate to ecological functions like resource exploitation is receiving more attention (Retana et al., 2015, Silva, 2010, Weiser and Kaspari, 2006). I sought to understand how ant functional traits vary across feeding guilds within NNFR ant community. Omnivorous ants had the largest

variation in body size ranging from ants with small to large body sizes. However, all ants with large body size and long legs within NNFR were omnivorous (Figure 2.11). This indicates that omnivorous ants within NNFR can occupy and forage in both structurally simple and complex habitat types owing to the variation in body size. They are known to have larger bodies than predators, and those with small body size tend to have longer limbs and larger eyes than predatory ants (Weiser and Kaspari, 2006). As omnivorous ants in NNFR are not restricted to any particular habitat, they may be able to contribute significantly to ecosystem functions through their exploitation of plants, impact on arthropod community and on surrounding soil across the whole area.

Generalists, carnivores and predatory ants all had negative PCA loadings on the first dimension of the PCA indicating small body size and shorter leg length than omnivorous ants. It is expected that ants belonging to these guilds would occupy and forage mainly in structurally complex and closed habitats such as forest habitat. However, some generalists and predators with small body size occurred in structurally simpler and open grassland habitat. They might have evolved strategies to avoiding desiccation in more open grassland habitat by foraging when it is cooler (Kaspari and Weiser, 2000) because small invertebrate are prone to desiccation in such open habitats (Kaspari, 1993, Kaspari and Weiser, 2000).

Specialized predatory ants usually have small body size (Hölldobler and Wilson, 1990), and some have small eyes (Weiser and Kaspari, 2006). Predatory ants in NNFR had smaller body size and eyes than omnivores (Figure 2.10). Ground dwelling ants that forage as specialized predators have been shown to generally have reduced eyes and in some species no eyes (Brown Jr, 2000, Delabie et al., 2000). One such specialized predator species *Dorylus emeyri* sampled at very low frequency in NNFR had no eyes. The second dimension of the PCA showed that predator ants within NNFR had longer mandible lengths in relation to their body size while omnivorous ant had shorter mandible lengths in relation to their body size. This makes sense because longer mandibles enable predatory ants to forage more efficiently by allowing them to forage for larger prey (Weiser and Kaspari, 2006). The small body size and leg length of predator ants within NNFR mean that they

might be able to forage more efficiently in specific habitats such as the more heterogeneous forest, where their impact on arthropod community is likely to be significant compared to the edge and grassland habitats. Since the grassland and the edge habitats had the highest ant species richness and diversity, it may be that the impact of predation by carnivorous ant species within in NNFR is lower in these habitats.

2.8.8 Conclusion

It is apparent that like most montane landscapes, there is low ant species richness and diversity in NNFR, which may be driven by the climatic conditions of which temperature is the most significant factor. Additionally, ant assemblages had similar foraging guilds represented by two main guilds- generalist omnivores and generalist carnivores. Omnivorous ants were the most abundant group represented. Functional traits of NNFR ant community varied across habitats. Differences in functional trait composition within a group/taxa means they would be capable of performing diverse functions within ecosystems (Eviner and Chapin III, 2003, Lavorel and Garnier, 2002). Determining how ant functional traits vary along grassland-forest improves our understanding of the species assemblage and their biological interactions across along this gradient (Lavorel and Garnier, 2002). In this study, I found that ants which occurred in both grassland and edge habitat showed more variation in functional traits compared to ants in the forest habitat. This indicates that ecological functions of ants in both the grassland and edge habitat would be more diverse than the forest habitat where functions might be more specialised. It is important to investigate the nature of their interactions with plant diaspores and determine if they include seed dispersal, which can aid natural forest regeneration, or whether they are antagonistic such as seed predation which hinders regeneration. This will be explored in the next chapter.

Appendix

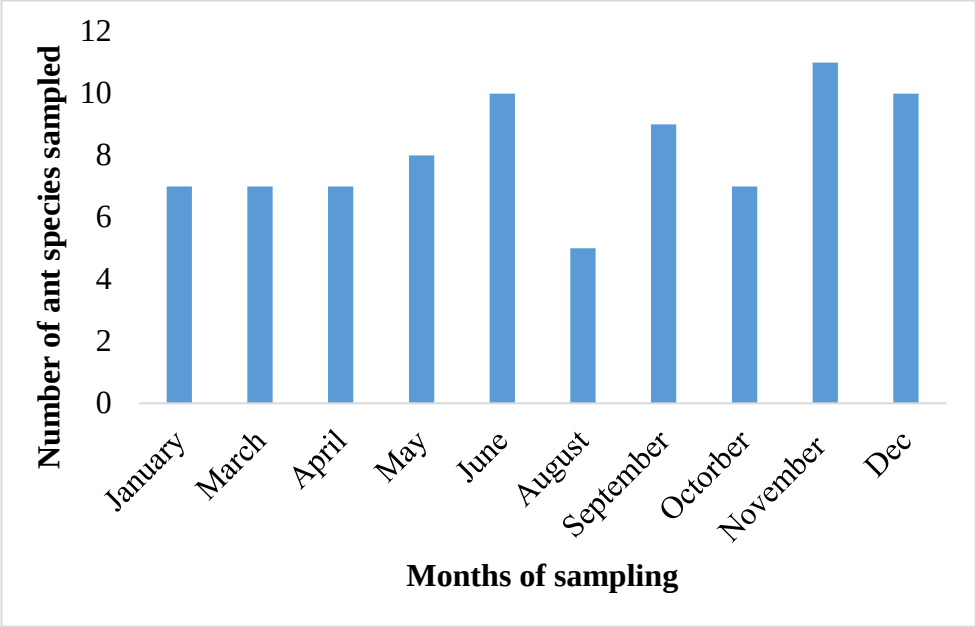

Figure 2.13: Ant species richness across 10 months of pitfall sampling

Chapter 3: Ant-diaspore Interactions in a West African Montane Forest: Mutualistic, Antagonistic or Both?

Abstract

In most tropical ecosystems, ground dwelling ant species are abundant and as such a good number of these species commonly interact with fallen seeds and fruits (diaspores). Through these interactions, they can affect the structure and function of plant community, but these effects often depend on the nature of interactions. So far, there are no reports on the nature of and significance of ant-diaspore interactions in West Africa and Nigeria. In this study, I present the first report on ant-diaspore interactions in an unexplored (in terms of ant-diaspore interactions) Afro-montane landscape. I looked out for opportunistic ant diaspore interactions along established transects encompassing three different habitats (grassland, edge and forest) within the reserve and carried out diaspore removal experiments using available small diaspores (weighing ≤ 1 g) that ants are likely to carry. Two ant species (*Pheidole* sp.1 and *Myrmicaria opaciventris*) were observed interacting with non-myrmecochorous diaspores of 10 plant species across nine families. Both ant species were small ants with body size < 0.2 mm. Observed interactions include cleaning of large diaspores (≥ 0.34 g) on the spot and removal of small diaspores (≤ 0.34 g). Diaspores of four plant species were cleaned on the spot while diaspores of six plant species were moved. Seventy percent of moved diaspores were taken under leaf litter while 30% were taken into ant nests. Ants moved diaspores of three edge tree species: *Trema orientalis, Bridelia specisosa* and *Harungana madagascariences,* and three species of forest trees: *Celtis gomphopylla, Polyscias fulva* and *Zanthoxylum leprieurii*. This study shows that ant-diaspore interactions in an Afro-montane forest in Nigeria involve fewer ant species than other in tropical ecosystems where interactions involving over 20 ant species in a single location have been reported. Nevertheless, the interactions recorded here can potentially have positive benefits through dispersal and diaspore cleaning which is known to reduce diaspore mortality. Potential negative effect of ant-diaspore interaction in this area could be the involvement of some of these ants as seed predators.

3.1 Introduction

Ants are ubiquitous and abundant in most terrestrial ecosystems around the world and influence many ecological processes (Folgarait, 1998, Frouz and Jilková, 2008). These include altering chemical properties of the soil (Beattie and Culver, 1983, Wagner et al., 2004), soil aeration (Benckiser, 2010) and facilitating seed germination and seedling establishment through their interactions with diaspores (diaspores being defined as fruit or seed as a unit of dispersal) (Brener and Silva, 1996, Christianini et al., 2007, Passos and Oliveira, 2003). Consequently, ants may substantially affect plant population and community structure (Zelikova et al., 2011).

Tropical forests are characterised by a high richness and diversity of plant species (Mittermeier et al., 1999) that produce a wide array of fleshy diaspores that are ingested and dispersed by vertebrate frugivores (Jordano, 2000). However, due to habitat loss and degradation in most of the world's forests (Brooks et al., 2002, Mittermeier et al., 1999), there has been a corresponding decline among populations of vertebrate seed dispersers (Brodie et al., 2009) and this negatively affects plant species that rely on their dispersal services. Hence, other animal groups less affected by habitat degradation could become more important as primary, or secondary dispersers, especially of small diaspores that have been partly processed and dispersed by frugivorous birds (Christianini and Oliveira, 2010).

Ground dwelling ants are mostly generalist in their foraging behaviour, feeding on a wide variety of food sources (Hölldobler and Wilson, 1990). They are, therefore, likely to encounter and exploit diaspores (Pizo and Oliveira, 2000) which are very abundant in tropical ecosystems (Lambert et al., 2005, Pizo and Oliveira, 2000). Indeed, ants have been observed interacting with the diaspores of herbaceous plants (Handel et al., 1981, Ness et al., 2009, Retana et al., 2004), shrubs (Boyd, 1996, Boyd, 2001) and trees (Passos and Oliveira, 2004, Pizo and Oliveira, 1998). The nature of these interactions is important as it determines whether or not they have a positive effect on the fitness of the plants involved (Zelikova et al., 2011).

3.2 Antagonistic ant-diaspore interactions

Antagonistic ant-diaspore interactions are those in which ants benefit at the expense of the plant species involved, and diaspore fitness and survival are negatively affected (Arnan et al., 2012, Bronstein et al., 2006). A common example of this is predation which involves ants harvesting and depositing diaspores in their nests where they are later consumed (Levey and Byrne, 1993, Retana et al., 2004). In this way, ants can significantly affect the diversity and succession of plant communities (Gorb and Gorb, 2003). In Australia, for example, where seed harvesting ants can be found in all major habitat types (Hulme et al., 2002) they have been shown to limit the recruitment of species that they prey upon (Wandrag et al., 2013). When seed-eating ants were eliminated from an area of South East Australia, a significant increase was observed in the seedling densities of *Eucalyptus baxteri* (Andersen, 1987).

Plants may, however, benefit from ‘predation' when the ant predator becomes satiated and abandons collected diaspores in their nest. Such diaspores then have an improved chance to germinate away from other predators (Beattie et al., 2002, Crawley, 2000, Hulme et al., 2002). In such cases, an antagonistic interaction becomes beneficial to the plant species involved.

3.3 Beneficial interactions

Benefits are usually derived from mutualistic interactions (Beattie, 1985) in which ants get a food reward from diaspore exploitation and plant benefit through increased germination success and seedling recruitment (Oliveira et al., 1995). The most widely studied mutualistic ant-diaspore interaction involves seeds with lipid-rich appendages known as elaiosomes (Beattie and Culver, 1981, Gorb and Gorb, 2003). Ants remove elaiosome-bearing seeds from the base of parent plants or from bird faeces to their nests where the elaiosome is consumed, and the seed is discarded unharmed within or outside the nest (Gorb and Gorb, 2003, Gove et al., 2007). This ant-mediated seed dispersal is described as myrmecochory, and the diaspores involved are referred to as myrmecochorous.

Recently, interactions between ants and non-myrmecochorous diaspores, i.e. diaspores without elaiosomes, have received more attention in the Neotropics and are reported to be beneficial to the plants involved (Christianini et al., 2007, Oliveira et al., 1995, Retana and Cerdá, 2000).

The main interactions reported are seed dispersal, and the removal of fruit pulp and fleshy appendages from seeds (seed cleaning) (e.g. Christianini et al., 2007). The observed benefits derived by plant diaspores from these interactions include: i) the reduction of seed mortality due to fungal growth, and increased germination success when ants "clean" diaspores (Oliveira et al., 1995, Pizo and Oliveira, 1998), ii) enhanced germination following transport of diaspores to ant nests, which may provide suitable microsites for germination (Christianini and Oliveira, 2010, Gorb and Gorb, 2003, Leal et al., 2007, Passos and Oliveira, 2003).

There is, however, a huge regional imbalance in the study of ant-diaspore (non-myrmecochorous) interactions, with most studies concentrated in the Neotropics. A few studies have been conducted in the Mediterranean region, but such interactions have remained unexplored in the Afrotropics. Hence, the question of what kinds of interactions occur between ants and non-myrmecochorous diaspores in Afrotropical forests and whether they have similar ecological consequences as having been reported in other areas, needs to be addressed. This is important because these forests harbour plant communities comprising a wide range of diaspore types and many ant species and are particularly affected by degradation and declines in frugivorous vertebrate dispersers (Effiom et al., 2013, Kuo et al., 2007)

The current study is, therefore, an exploration of ant-diaspore interactions within Ngel Nyaki Forest Reserve (NNFR), a montane forest in the West African country of Nigeria (for location and site details, see chapter 2). It is characterised by high altitude (1400- 1600 m above sea level) and a diversity of plant species, many of which are endemic (Barnes et al., 2014, Chapman et al., 2001). It has also been observed, that diaspore size tends to decrease with increasing altitude (Chapman et al., 2016, Friis, 1992) and there is an abundance of small diaspores in NNFR suggesting that ant-diaspore interactions may be frequent here. During a study on the role of seed dispersal, predation and drought in the restoration of NNFR, Roselli

(2014) observed interactions between ants and some small diaspores. However, the ant species interacting with these diaspores, the nature of the interactions, and how they may affect seed germination and seedling recruitment in this important montane forest are yet to be determined. Obtaining such information is the primary aim of this chapter.

3.4 Objectives

The specific objectives of this study were to:

1. Determine how ants interact with diaspores in this West African montane landscape and whether such interactions are influenced by habitat differences.
2. Identify ant species that transport diaspores, and the plant species which benefit from such interactions
3. Identify microhabitat characteristics that might affect such interactions

The null hypotheses were as follows:

- Ants do not clean, disperse, or bury fallen diaspores within NNFR.
- Diaspore removal and removal distance are not affected by the interacting ant and plant species
- There is no spatial or temporal variation in diaspore removal rate by ant species in NNFR.
- Microhabitat characteristics such as litter depth, tree density, and canopy cover have no effect on removal and dispersal of diaspores by ants.

3.5 Methods

3.5.1 Study Design

The study was carried out at three sites within NNFR (detailed description in Chapter 1). Sites were separated by a distance of between 0.5 to 1 km and included core forest, forest edge part of a fenced-off regenerating grassland adjacent to the core of the forest reserve (Figure3.1).

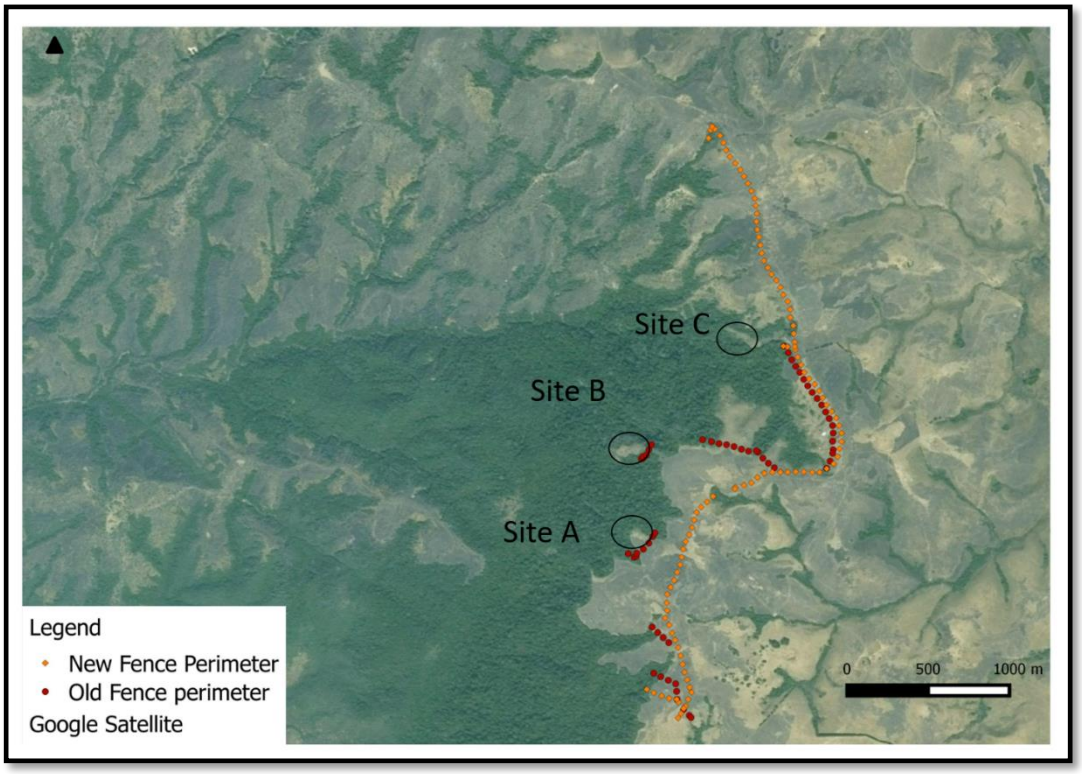

Figure 0.1: Map of Ngel Nyaki Forest Reserve with study sites marked (Adapted from Google Maps 2016). Black circles show study sites A, B and C.

Investigations were conducted along transects (described in Chapter 2) that ran through the three habitats; core forest (forest), forest edge (edge), and grassland. The forest habitat was defined as being at least 41 m from the outermost canopy tree (0 m) to 160 m into the forest; edge habitat was defined as beginning from 40 m into the forest, to the outermost canopy trees at 0 m, and up to 19 m into the grassland. Additionally, plant species such as *Paullinia pinnata* and *Trema orientalis* which are known to be edge specialists were used to delineate the edge habitat. Grassland habitat began 20 m from the outermost canopy trees at the forest edge, up to 160 m into the surrounding grassland (figure 3.2). Observations and experiments were conducted between July 2015 and September 2017.

3.5.2 Reconnaissance surveys for ant-diaspore interactions

Reconnaissance surveys for opportunistic ant-diaspore interactions were carried out monthly between June 2015 and September 2015 along transects. This was done by walking along established transects between 0730 hrs – 1300 hrs at a slow pace

(1km/hour) and searching on the ground approximately 2 m on either side of the transect. If an ant was spotted interacting with a diaspore (contact with the surface of a diaspore and or moving the diaspore), the ant species, plant species, and type of interactions were recorded after the method of (Pizo and Oliveira, 2000).

3.5.3 Diaspore removal experiment

Diaspore removal experiment involved small diaspores (fruits and seeds) that weighed ≤ 1 g. This falls within the limits of seeds that an individual ant might move (Passos and Oliveira, 2003, Pizo and Oliveira, 2001). Diaspores were from 13 plant species whose diaspores were available at the time of the experiment. The species names, plant family, diaspore weight, and primary means of dispersal are shown in (Table 3.1).

Fruits of each tree species were collected from five different individual parent plants and seeds were manually removed from collected fruits, washed and sun-dried for one hour. Experiments were carried out between 0730 hrs and 1300 hrs.

Table 0.1: Plant species and diaspore types used for diaspore removal experiment**.**

Plant species	**Family**	**Diaspore type**	**Wet weight (g)**
Allophylus africanus	Sapindaceae	Fruit	0.60
		Seed	0.07
Bridelia speciosa	Euphorbiaceae	Fruit	-
		Seed	0.07
Celtis gomphophylla	Cannabaceae	Fruit	0.21
		Seed	0.01
Antidesma sp	Ebenaceae	Fruit	0.11
		Seed	0.02
Harungana madagascariensis	Hyparicaceae	Fruit	-
		Seed	-
Macaranga occidentalis	Euphorbiaceae	Fruit	0.04
		Seed	0.03
Maesa lanceolata	Myrsinaceae	Fruit	-
Polyscias fulva	Araliaceae	Fruit	0.07
		Seed	0.01
Psorospermum aurantiacum	Guttifereae	Fruit	0.56
		Seed	0.05
Psychotria sp.	Rubiaceae	Fruit	0.23
		Seed	0.05
Rauvolfia vomitoria	Apocynaceae	Fruit	0.56
		Seed	0.09
Trema orientalis cleaned	Cannabaceae	Seed	0.005
		Fruit	0.04
		Cleaned seed	0.01
Zanthoxylum leprieurii	Rutaceae	Seed	0.25

At each site, two 320 m transects starting from the grassland, terminating in the core forest, and separated by a distance of 20 m were established. Thirteen seed depots were placed along each transect a forest-grassland gradient, starting right at the forest edge (0 m from the outermost forest canopy) and going in both directions (into the forest and grassland) at 5 m, 10 m, 20 m, 40 m, 80 m and 160 m. This design (Figure 3.2) was used to allow for comparisons in ant-diaspore interactions across the different habitats (Gallegos et al., 2014).

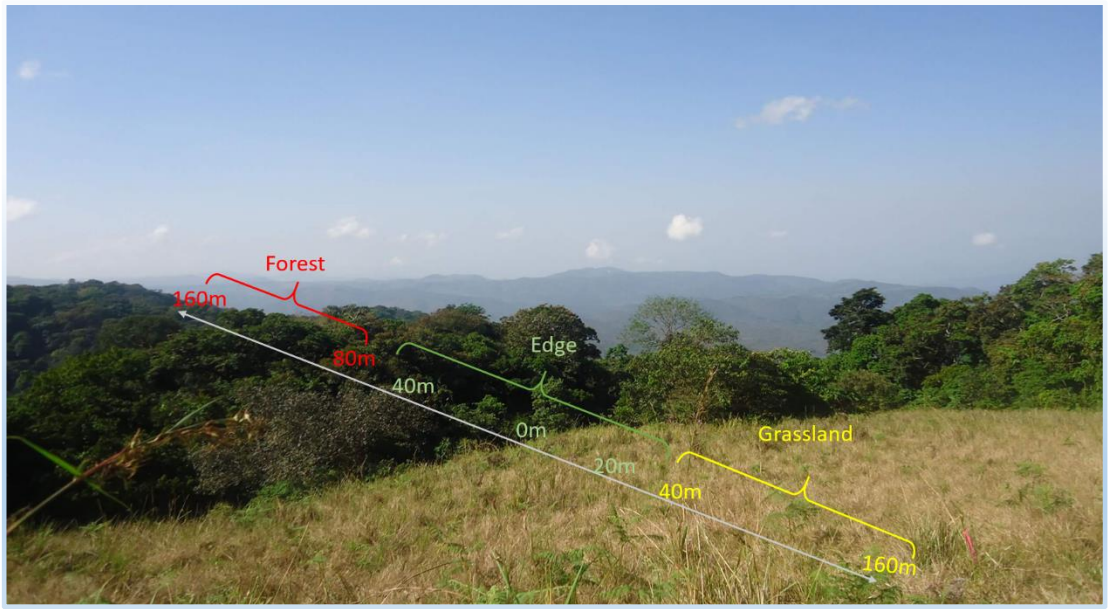

Figure 0.2: Experimental set-up along forest to grassland gradient showing positions of seed depots.

3.5.4 Focal observations of ant-diaspore interactions

Each depot comprised of two fruits and seeds of the same plant species placed on a piece of white filter paper 1 m off the transect (Figure 3.3) (Christianini et al., 2012). The white filter paper was to facilitate visibility and was not expected to influence ant behaviour as demonstrated in other studies (Christianini et al., 2007, Passos and Oliveira, 2004).

Figure 0.3: Experimental diaspore depot for observing ant-diaspore interactions

Observations were carried out between 0700hrs - 1300hrs. Two observers positioned 0.5 m on either side of each depot monitored depots for 15 minutes, during which all ant-diaspore (seed/fruit) interactions were recorded. (Christianini et al., 2012). Ant behaviour was recorded and classified as (1) removed diaspore to the nest, (2) consumed diaspore on the spot, (3) no removal/interaction (Christianini and Oliveira, 2009).

To determine if body size influences ant behaviour towards diaspores, ants observed interacting with diaspores were classified according to their total body size as; small (< 0.2cm), medium (0.2cm -1.0 cm) or large (>1cm) (Arnan et al., 2010). Ants that removed diaspores were followed from the point of interaction to the nest or other location where diaspores were deposited. Nests where diaspores of *T. orientalis* were deposited, were marked and observed weekly for four months to determine if diaspores taken into nest would germinate eventually or be discarded outside the nest. *T. orientalis* was used for this observation due to the availability of the

diaspores throughout diaspore removal experiement. Distance from the experimental depot to where diaspores were deposited (dispersal distance) was measured.

Also, at any time during the experiment, each opportunistic ant-diaspore interaction observed on forest trails on the route to the experimental sites or elsewhere within the reserve, was recorded and the nature of interactions noted. Samples of ant species observed interacting with diaspores during the experiment were collected and taken back to the School of Biological Sciences, the University of Canterbury, New Zealand for identification. An export permit was not required to bring ant samples from Nigeria to New Zealand. Identifications to genus level were made using taxonomic keys produced by Bolton (1994).

3.5.5 Effect of microhabitat, diaspore type, and alternative food sources on diaspore removal by ants

To investigate the effect of microhabitat characteristics; litter depth, the presence of trees, and canopy cover on ant-diaspore removal, a separate diaspore removal experiment using only diaspores of *T. orientalis* was conducted using the same set-up as the previous one. However, at each experimental depot, litter depth was measured using a meter rule, the number of woody plant species within a perimeter of 2 m was recorded, and canopy cover at each was assessed and categorised as open, partly closed or closed. Canopy cover was categorized as 'open' (0- 20% canopy cover); 'partly closed' (21-64% canopy cover), and 'closed' (65-100% canopy cover). The frequency of ants foraging on other food sources (arthropods) was noted during focal observation of each experimental depot. Three diaspore types were used in this experiment to investigate if ant-diaspore removal was predation or not: (i) fruits, (ii) seeds from which fruit pulp had been removed, and (iii) cleaned seeds devoid of any fibrous or fleshy tissue (remaining fleshy appendages and fibrous tissue were manually removed the seeds- Figure E in general appendix). Removal of diaspores that lacked any fleshy tissue was taken to be predation

3.6 Data analysis

Data were compiled into an Excel spreadsheet and analysed using R version 3.2.4 with *lme4* and *car* packages. For all analysis, a p-value less than 0.05 was considered significant.

3.6.1 Spatial and temporal variation on ant diaspore removal

A generalised linear mixed-effect model (GLMM) with binomial error distribution (to account for binomial response variable) was used to assess spatial and temporal variations in ant diaspore removal. The response variable was a combination of diaspore "removed" and not removed," while independent variables were fixed effects that included ‘habitat’, ‘season’, ‘ant species’, ‘plant species’, and their two-way interactions. The random effects included in the models were ‘transect ID’, ‘site’ and ‘depot ID’.

3.6.2 Effect of ant species and plant species on diaspore dispersal distance

A GLMM with Poisson error distribution (due to count response variable) was used to assess how ant species and plant species affect the diaspore removal distance. The response was the distance moved, and the independent variables were the fixed effects and included ‘ant species’, ‘plant species’ and their interactions as fixed effects, while random effects were ‘transect ID’, ‘depot’ and ‘observation ID’.

3.6.3 Effect of microhabitat characteristics and diaspore type (seed or fruit) on diaspore removal

A GLMM with binomial error distribution (to account for the binomial response variable) was used to assess the effect of microhabitat characteristics on ant-diaspore removal using the data from the *T. orientalis* removal experiment. The response variable was diaspore "removed" and not removed," and the independent variables were fixed effects which included ‘canopy cover’, ‘litter depth’, ‘number of woody plant species’, diaspore type (fruit, seed or cleaned seed)’ and their two-way interactions. Random effects were ‘transect ID’ and ‘depot ID’.

3.6.4 Effect of microhabitat characteristics on the number of diaspores removed

A GLMM with a Poisson error distribution (because the response variable was count data) was used to analyse the effects of canopy cover, litter depth, foraging frequency on other food sources (arthropod) on the number of diaspores moved by ants. The response was the ‘number of diaspores moved’, and the independent variables were the fixed effects which included ‘canopy cover’, ‘litter depth’, ‘foraging frequency of

other arthropods' and their interactions, with 'transect ID' and 'depot ID' as random effects.

All models were compared using one-way ANOVA and factors that had the least significant effects were removed. Akaike Information Criteria (AIC) were used to choose the best fit model (Crawley, 2002a, Symonds and Moussalli, 2011). When models were not significantly different, the one with the fewest factors were chosen as the best fit model. The *blmeco* package in R was used for model validation by checking for overdispersion. An observational-level random effect was included to models to account for overdispersion (Harrison, 2014).

3.7 Results

3.7.1 Ant-diaspore interactions

Very few ant-diaspore interactions were observed during the reconnaissance survey of opportunistic ant-diaspore interactions with just 18 encounters recorded in four months (representing 16 hours of reconnaissance survey). Ants interacted with entire diaspores, fruit pulp, seed appendage (such as arils of *P. pinnata*) and seeds of the plant species. Two types of interactions; removal of diaspores, and consumption of diaspore appendages on the spot were observed and involved two ant species (*Myrmicaria opaciventris* and *Pheidole* sp.1) belonging to the sub-family Myrmicinae, and six plant species as summarised in Table 3.2.

Table 0.2: Opportunistic ant-diaspore interactions recorded during reconnaissance survey in Ngel Nyaki Forest Reserve

Plant species	**Ant species**	**Diaspore type/portion**	**Interaction type**
Psorospermum aurantiacum	*Pheidole sp. 1*	Whole fruit	Moved
	M. opaciventris	Fruit pulp	Eaten on the spot
Paullinia pinnata	*Pheidole sp. 1*	Seed aril	Eaten on the spot
	M. opaciventris	Seed aril	Eaten on the spot
Syzygium macrocarpa	*Pheidole sp. 1*	Fruit pulp	Eaten on the spot
Trema orientalis	*Pheidole sp. 1*	Fruit and seed	Moved
	M. opaciventris	Fruit and seed	Moved
Chionanthus africanus	*Pheidole sp. 1*	Fruit pulp	Eaten on the spot
Zanthoxylum leprieurii	*M. opaciventris*	Whole fruit	Moved

During the survey totalling 16 hours, *Myrmicaria opaciventris* was observed interacting with diaspores only in the grassland and edge habitats but not in the forest habitat while *Pheidole* sp.1 was observed interacting with diaspores in the three habitat types (Figure 3.4).

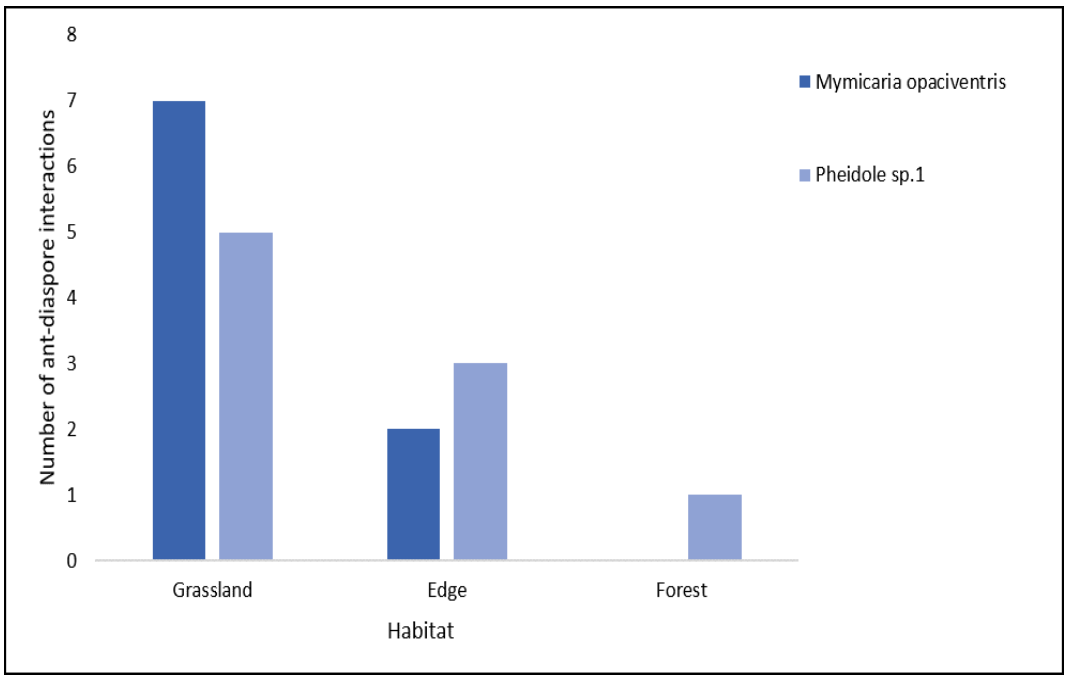

Figure 0.4: Number of opportunistic ant-diaspore interactions and the ant species involved observed during reconnaissance surveys across habitats in Ngel Nyaki Forest Reserve.

3.7.2 Spatial and temporal variation in diaspore removal

In the diaspore removal experiment, habitat did not affect diaspore removal by ants significantly. However, season, ant species, and plant species (Figure 3.5) had significant effects on diaspore removal (Table 3.3). The mean frequency of diaspore removal was greater during the wet season (0.29 ± 0.02 s.e) than the dry season (0.06 ± 0.01s.e).

Table 0.3: Results of the generalised linear mixed model with binomial distribution showing the effect of habitat, season, ant species and plant species on diaspore removal. Significance is denoted by an asterisk at $p \leq 0.05$

Sources of variation	Chisq	df	p-value
Habitat	3.2	2	0.19
Season	4.59	1	< 0.05*
Ant species	51.2	2	< 0.0001*
Plant species	30.1	4	< 0.0001*

3.7.3 Effect of ant and plant species on removal distance

Dispersal distances ranged from 0.2 cm to 1.24 m with a mean of 28.6 cm, and median of 24cm. Of all diaspores species moved by ants, *T. orientalis* had the highest mean dispersal distance of 14.04 cm (Figure 3.5).

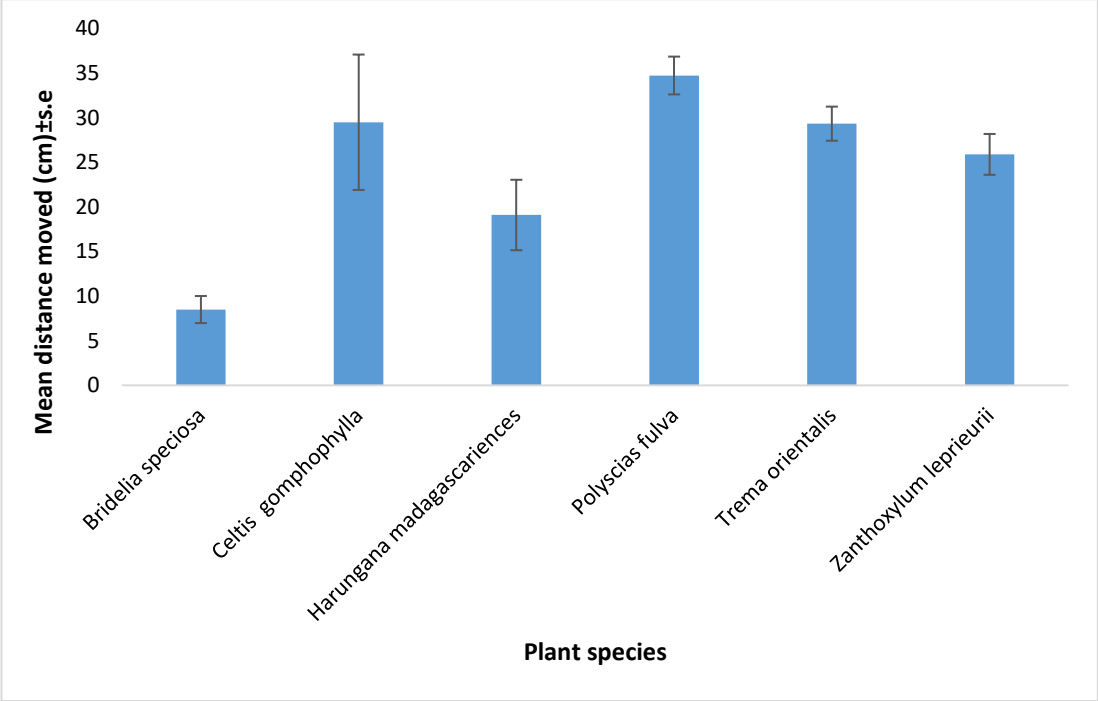

Figure 0.5: Mean removal distance of plant species moved by ants during diaspore removal experiment within Ngel Nyaki Forest Reserve. *B. speciosa* (n = 12), *Celtis gomphophylla* (n = 9), *Harungana madagascariensis* (n = 20) , *Polyscias fulva* (n = 29), *Trema orientalis* (n =146) and *Zanthoxylum leprieurii* (n = 33).

Dispersal distance did not differ significantly across habitats and between ant species but was significantly different among plant species (Table 3.4)

Table 0.4: Results of the generalised linear mixed model with Poisson distribution showing the effect of habitat, ant species and plant species on diaspore removal distance. Significance is denoted by an asterisk at $p \leq 0.05$

Source of variation	**Chisq**	**df**	***p*-value**
Habitat	0.02	2	0.98
Ant species	0.08	2	0.76
Plant species	22.5	5	< 0.001*

3.7.4 Overall diaspore removal by *Pheidole* sp. 1 and *M. opaciventris*

Nineteen percent (19%) of the 1315 diaspores used in the diaspore removal experiment were removed by ants within 15 minutes of being placed on the ground. Not all diaspore species were equally attractive to the ants, with significantly more diaspores of *Z. Leprieurii* and *T. orientalis* being removed than those of *B. speciosa, C. gomphophylla, P. Fulva* or *H. madagascariensis* (Figure 3.6).

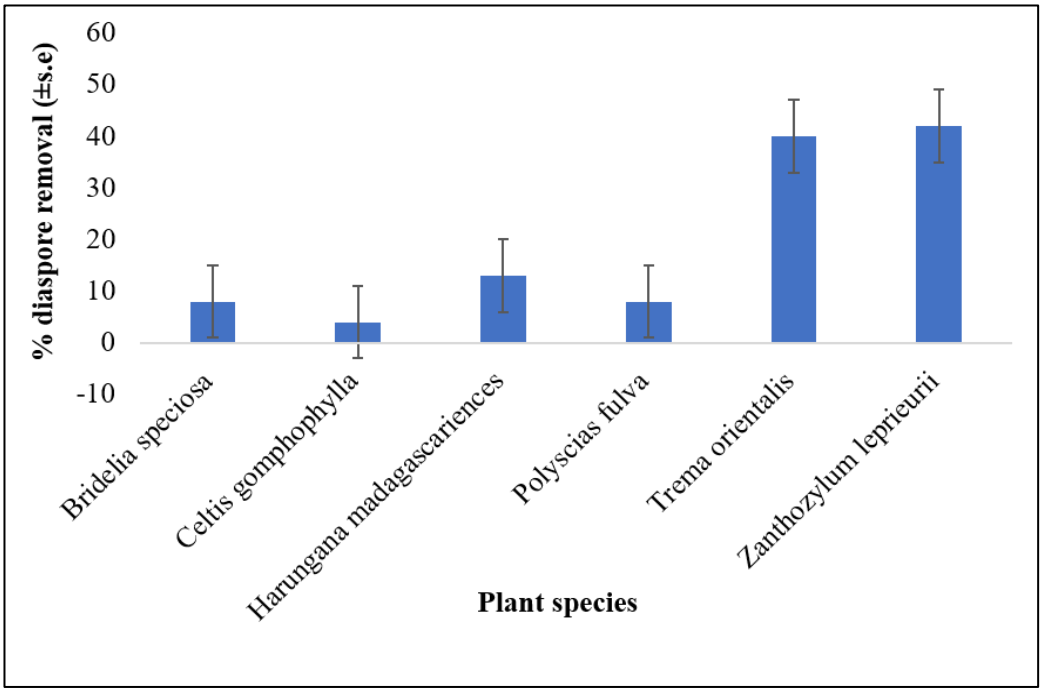

Figure 0.6: Proportion of diaspores of each plant species moved by ants during diaspore removal experiment within Ngel Nyaki Forest Reserve. *B. speciosa* (n = 156), *C. gomphophylla* (n = 251), *H. madagascariensis* (n = 155), *P. fulva* (n = 368), *T. orientalis* (n =307) and *Z. leprieurii* (n = 78).

Seventy percent (70%) of diaspores were carried by ants into leaf litter and were effectively "lost" to the observer, while the remaining 30% could be traced all the way into ant nests.

Pheidole sp.1 removed a greater proportion of the diaspores (74%) than *M. opaciventris*, which removed 26%. Of those moved by *Pheidole* sp.1, 36% were

observed being moved into nests while 64% were lost to sight within leaf litter. Only 12% of diaspores moved by *M. opaciventris* were followed to nests, and 88% disappeared into leaf litter (Figure 3.8).

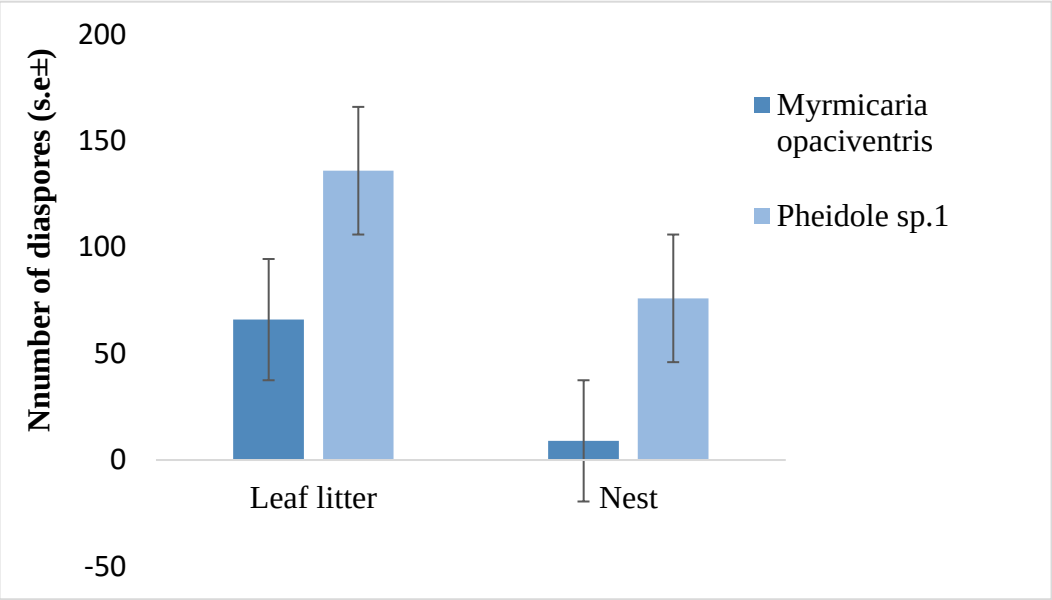

Figure 0.7: Diaspore removal by *Myrmicaria opaciventris* and *Pheidole* sp.1 and location of deposition.

The ant nest locations into which seeds of *T. orientalis* were deposited were monitored for four months following the experiment. During this period 21 *T. orientalis* seedlings were counted growing from within seven of such nests.

3.7.5 Effect of microhabitat characteristics and diaspore type (seed or fruit) on diaspore removal

In the experiment to determine microhabitat effects on diaspore removal using *T. orientalis* as the model species, diaspore type (fruit, seed or cleaned seed- stripped of all fleshy appendages) had a significant effect on diaspore removal, with ants significantly removing more seeds with fibrous/fleshy tissue than fruit or cleaned seeds (Table 3.5). Litter depth and the interaction between canopy cover and litter depth also affected removal of *T. orientalis* diaspores significantly. Litter depth had a negative effect on diaspore removal, and diaspores were less likely to be removed

under high litter and closed canopy (Appendix 3.1). However, canopy cover alone and the presence of woody plants had no significant effect on diaspore removal (Table3.5)

Table 0.5: Results of the generalised linear mixed model with binomial distribution showing the effect of canopy cover, diaspore type, woody plants, litter depth (cm) and the interaction between canopy cover, litter depth (cm) on the number of diaspores removed. Significance is denoted by stars at p = 0.05

Sources of variation	Chisq	df	p-value
Canopy cover	3.8	2	0.14
Diaspore type	42.2	2	< 0.0001*
Number of woody plants	0.02	1	0.86
Litter depth (cm)	7.81	2	< 0.01*
Canopy cover: Litter depth (cm)	1.30	3	< 0.05*

3.7.6 Effect of microhabitat characteristics on the number of *T. orientalis* diaspores removed

Canopy cover, litter depth, the frequency of ant foraging frequency on arthropods, and the interaction between canopy cover and litter depth (cm), significantly affected the number of diaspores removed by ants. The proportion of diaspores removed in relation to canopy cover is shown in Figure 3.7. Litter depth had a negative effect on the number of diaspores removed. More diaspores were removed in open canopy/ shallower litter and partly closed canopy and shallower litter than in closed canopy/ shallower litter. In contrast, the number of woody plant species near the depot did not affect the number of diaspores moved (Table 3.6).

Table 0.6: Results of the generalised linear mixed model with Poisson distribution on the effect of canopy cover, diaspore type, woody plants, litter depth (cm), frequency

of ant foraging on arthropods, and the interaction between canopy cover and litter depth (cm,) on ant-diaspore removal. Significance is denoted by an asterisk at $p \leq 0.05$.

Sources of variation	Chisq	df	*p*-value
Canopy cover	6.4	2	< 0.05*
Number of woody plants	0.17	1	0.67
Litter depth (cm)	7.34	2	< 0.01*
Frequency of foraging on arthropods	28.0	1	< 0.0001*
Canopy cover: Litter depth (cm)	9.15	3	< 0.05*

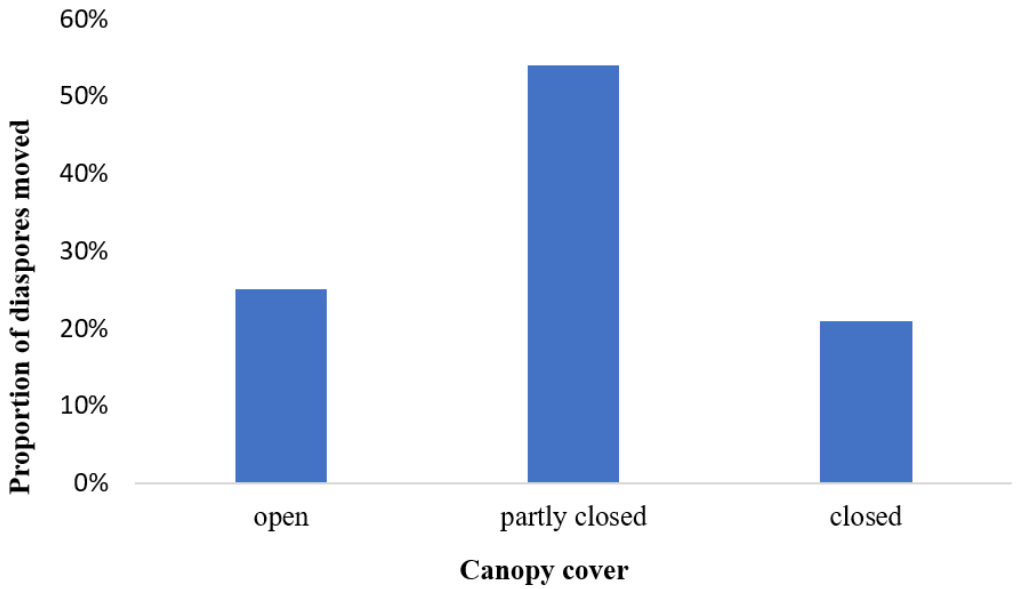

Table 0.7: Proportion of *Trema orientalis* diaspores moved by ants under varying degrees of canopy cover.

3.8 Discussion

Up to 90% of diaspores produced by plants in tropical forests are adapted for dispersal by vertebrate species such as mammals and birds (Holbrook and Smith, 2000, Jordano, 2000), and much attention has been given to vertebrate-diaspore interactions involving large bodied frugivores (Chapman and Russo, 2007, Forget, 1990, Jordano, 2000, Levey et al., 2002). However, elucidating the contributions of small bodied dispersers, is important particularly in seconday dispersal of small diaspores. They can serve as substitute dispersers especially in disturbed landcapes with declining vertebrate dispersers. Interactions between ants and non-myrmecochorous diaspores are unreported in Afrotropical lowland and montane forests. Such interactions are common in Neotropical forests and have been shown to affect the structuring of plant communities and contribute to regeneration in degraded habitats (Gallegos et al., 2014). I investigated ant-diaspore interactions in Ngel Nyaki Forest Reserve, the largest montane forest in Nigeria, and report on such interactions within the Afrotropics for the first time, considering whether they can contribute to regeneration in the study area.

3.8.1 Frequency of ant-diaspore interactions-reconnaissance survey

A reconnaissance survey to detect opportunistic ant-diaspore interactions yielded only 18 such interactions in 16 hours of observations (1.25 interactions /hour of effort). This is likely to be a gross underestimation due to poor visibility, especially within the forest habitat, and the small size of both ants and diaspores. Diaspore removal experiments using diaspore 'depots' clearly provide a better approach to studying these interactions.

3.8.2 Types of ant-diaspore interactions

The combination of opportunistic observations and diaspore removal experiments revealed that of 17 ant species identified in NNFR, only two; *Pheidole* sp.1 and *Myrmicaria opaciventris,* interacted with diaspores belonging to a total of ten non-myrmecochorous plant species. Diaspores of six plant species out of 12 used for diaspore removal experiment were moved by ants and they were opportunistically observed exploiting on the spot diaspores of four plant species. This corroborates

reports from the Neotropics that ants commonly interact with non-myrmecochorous diaspores of several plant species (Christianini et al., 2012, Kaspari, 1993, Levey and Byrne, 1993, Pizo and Oliveira, 1998), but differs significantly from those reports in the small number (two) of ant species involved. For example, 41 ant species were found interacting with diaspores of 56 plant species in the Brazilian lowland Atlantic forest (Pizo and Oliveira 2003). The discrepancy may be explained in part, by the low ant species richness and diversity recorded in the site of the present study compared to the Neotropics, which contain some of the most diverse ant and plant assemblages on Earth (Corlett and Primack, 2011).

3.8.3 Ants and plant species involved in interactions

The finding that only two ant species (*Pheidole* sp. 1., *Myrmicaria opaciventris*) were interacting with diaspores in NNFR, was unexpected because at least four other morphospecies identified in the reserve belong to the genera *Camponotus* and *Lepisiota* (Chapter 2, Table 2.9), both considered to comprise mainly generalist omnivorous species (Bolton, 2007) whose diets are likely to include diaspores.

However, these two small myrmicine ants, *Pheidole 1* and *M. opaciventris*, with body sizes of < 0.2 mm, interacted with a wide range of diaspores species; nine trees and one liana. Only small diaspores, weighing ≤ 0.24g were moved, while fruit pulp and/or other fleshy seed appendages were consumed on the spot from diaspores that were apparently too large for them to move (Table 3.1). This implies that only small seeded plants are likely to benefit from ant-mediated dispersal in NNFR.

3.8.4 Diaspore removal rates by *Pheidole* sp. 1 and *Myrmicaria opaciventris*

Diaspore removal by *Pheidole* sp.1 and *M. opaciventris* differed significantly; *Pheidole* sp.1 accounted for 75% of all the diaspore removed during the study while *M. opaciventris* only removed 25%. This result may reflect differences in the diets of these two generalist omnivorous ant species. Although not much is known about *M. opaciventris* diet, a study in Cameroon found that while it is quite a generalist omnivore in its foraging, a significant proportion of its diet was made up of insects and it even robbed insect prey from raiding army ant columns (Kenne and Dejean, 1999). This study is the first to report its interactions with diaspores. In contrast,

many *Pheidole* species are known to be both generalist omnivores and even granivorous (O'Dowd and Gill, 1984, Pizo and Oliveira, 1998). This might explain why they removed more diaspores than *M. opaciventris*.

3.8.5 Ants in NNFR do not move diaspores far enough to avoid density-dependent effects

Previous studies have shown that diaspores moved farther from parent plants avoid intra-specific negative density-dependent (ISNDD) mortality (Janzen-Connell effect) (Connell, 1971, Janzen, 1970) and therefore, have an improved chance of germination and recruitment (Andersen, 1988, Cain et al., 2000, Gallegos et al., 2014, Nathan and Muller-Landau, 2000).

Ants may move diaspores from where they land undispersed on the ground below the parent tree, or move diaspores which have already been dispersed by primary dispersers, into safe microsites more suitable for germination and establishment (Christianini et al., 2007). If such movement is sufficient to remove or significantly reduce ISNDD effects, it could potentially play a significant role in improving seedling recruitment in Ngel Nyaki forest. However the ants in this study did not generally move diaspores across distances such that they would be removed from ISNDD; diaspores were moved on average 28.6 cm (range: 0.20 cm - 124 cm and median: 24 cm), a considerably shorter distance than the global estimated mean removal distance of 1.99 m recorded for ants and myrmecochorous diaspores (Gómez and Espadaler, 2013). Moreover Christianini et al., (2007) in a study of ant-interactions with vertebrate-dispersed diaspores in Brazil, found that ants moved diaspores up to 25 m with a mean removal distance of about 5 m. However, elsewhere, small ants, like those found in the current study, were shown to move diaspores shorter distances compared to ants with large body size (Gómez and Espadaler, 2013, Ness et al., 2004) and is associated with the fact that small ants often forage close to their nests.

As well as ant body size, the density and distribution of ant nests may affect dispersal distance (Andersen, 1988). Although ant nest density and distribution were not assessed during this study, the short distances that ants moved diaspores may be

indicative of high nest density, and during the focal observations ants were seen moving diaspores to nest locations not far from experimental depots.

Therefore, within NNFR ants are unlikely to contribute to increased seed germination and establishment by reducing ISNDD effects. However, they may be important for rearranging the seed shadow of diaspores primarily dispersed by vertebrates and other means, by moving them into more suitable microsites such as leaf litter and nest where diaspores can avoid desiccation (especially when the environmental conditions are not suitable) as shown by (Christianini and Oliveira, 2010, Giladi, 2006, Vander Wall and Longland, 2004). Such diaspores, if not consumed by the ants, would also avoid being predated upon by seed predators (Christian and Stanton, 2004, Christianini et al., 2007, Gorb and Gorb, 2003).

The secondary removal by ants of diaspores primarily dispersed by vertebrates (Christianini and Oliveira, 2013, Passos and Oliveira, 2002, Pizo and Oliveira, 1998) has been shown to increase plant recruitment (Christianini and Oliveira, 2009), even in degraded habitats (Gallegos et al., 2014). Whether or not this is the case in NNFR is uncertain. While 30% of the diaspores moved by ants during the diaspore removal experiment were observed to be deposited within ant nests (Figure 3.6), their eventual fate could not be established. The potential exists for ant removal to positively affect germination and establishment rates but I was not able to demonstrate this within the time constraints of this study.

3.8.6 Effect of habitat and season ant diaspore removal rates and distance in NNFR

Ant-diaspore removal and removal distances within NNFR did not differ among the three habitats (grassland, edge and forest). In contrast, studies elsewhere have found ant-diaspore removal rates and dispersal distances to vary with habitat (Leal et al., 2014, Zelikova and Breed, 2008). For example, removal by ants and dispersal distance tends to decrease with disturbance (Leal et al., 2014) or with the presence of invasive ants species (in disturbed habitats) and as such, invaded habitats have decreased dispersal distances compared to uninvaded habitats (Christian, 2001, Ness et al., 2004). The lack of variation in ant-removal rates and dispersal distances across the three habitat types within NNFR may be explained by the fact that the two same

ant species are responsible for removal within the different habitats. Variation in diaspore removal rates and removal distance across habitat have been shown in areas where diaspore interacting ant species composition differs across habitat (Gallegos et al., 2014, Majer, 1992). It could also be because the level of disturbance within these habitats has been reduced by the fencing-off and exclusion of anthropogenic activities such as grazing and fires (Roselli, 2014) which were the main disturbance threats facing the area. Hence diaspores may be removed by ants and transported to locations of deposition without interference.

Diaspore removal rates within NNFR varied between the wet and dry seasons, with more diaspores being moved by ants during the wet season. Previous studies elsewhere in the tropics have shown that diaspore availability is the main source of temporal variation in ant-diaspore interactions (Passos and Oliveira, 2004) and that this is highest during the wet season (van Schaik et al., 1993). Plants that can successfully disperse their diaspores into suitable microsites for germination during the wet season have a higher chance of recruitment than in the dry season because, seeds that germinate in this period are more likely to survive harsher environmental limitations associated with lower moisture available at the onset of the dry season (Rey and Alcantara, 2000, Verdú and Traveset, 2005). Thus, the higher abundance of fleshy diaspores within NNFR during the wet season as is found in most tropical ecosystems (van Schaik et al., 1993) appears to be the most plausible reason for the seasonal variation in ant-diaspore removal.

3.8.7 Effect of microhabitat, diaspore type, and alternative food sources on diaspore removal by ants

Habitat structure on a small spatial scale, can influence the probability of ant-diaspore interactions (Raimundo et al., 2004). Data from the current study shows that litter depth and the interaction between litter depth and canopy cover affected the rate of ant-diaspore interactions and the number of diaspores moved by ants within NNFR. More diaspores were moved by both small-bodied ant species in shallow rather than deeper litter (Figure 3.8). This is in contrast to a recent study on litter depth and ant foraging patterns in Costa Rica, which found that large ants were

constrained by deep litter while smaller ants were able to efficiently forage in it (Farji-Brener et al., 2004).

In addition, open canopy/shallower litter depth and partly closed canopy/shallower litter depth together appeared to have a positive effect on diaspore removal rate and the number of diaspores removed. This indicates that there might be a within-habitat spatial variation that affects diaspore removal. Spots with open/partly closed canopy and shallow litter appear to be more favourable for ant-diaspore interactions and removal in NNFR. Diaspores which land on more suitable spots within a habitat are more likely to be removed rapidly and avoid being predated upon by vertebrate predators such as rodents (Christian, 2001). Ant species richness and abundance were higher in the grassland and the edge habitat which has open and partly closed canopy, than the more closed forest habitat of NNFR (see chapter 2 for details). Although habitat had no significant effect on ant-diaspore removal, it can be inferred that more suitable patches might be available for ant diaspore removal in the edge and grassland habitats than the forest which is mostly closed canopy.

Diaspore removal was not affected by the density of woody plant species. The influence of tree density on ant-diaspore interactions has been shown to affect arboreal ant species (Passos and Oliveira, 2003). The fact that this study found only ground dwelling ants interacting with diaspores explains why density of woody plants did not affect diaspore removal by ants.

Seeds of *T. orientalis* were more likely to be removed by ants than its fruits and cleaned seeds, suggesting that *Pheidole* sp.1 and *M. opaciventris* may be acting either as secondary seed dispersers, seed predators, or both, as was found for *Messor bouvieri* and seeds of *Lobularia maritima* in the Mediterranean (Barroso et al., 2013). The fact that *T. orientalis* lacks any special adaptation (elaiosome) for ant dispersal and is primarily bird dispersed (Adinortey et al., 2013) supports this assertion. Both ant species moved diaspores into their nests or disappeared with diaspores under leaf litter. Because of this, it was not confirmed whether they consumed the diaspores or only removed fleshy portions attached to the diaspores. However, when seeds of *T. orientalis* were completely stripped of all flesh and fibrous tissue (cleaned seed), *M. opaciventris* largely ignored them, and on the three

occasions that it moved cleaned seeds, they were dropped a short distance from the point of removal. This suggests that *M. opaciventris* is not a seed predator of *T. orientalis* (and perhaps the other plant species it interacted with), and could function as a disperser. In contrast, *Pheidole* sp.1 moved all diaspore types including cleaned seeds of *T. orientalis*. Several species from the genus *Pheidole* are recognized as important seed harvesters in different ecosystems around the world (Levey and Byrne, 1993, O'Dowd and Gill, 1984, Pizo and Oliveira, 1998). It is possible that *Pheidole* sp.1 found in NNFR is a seed harvester that causes seed mortality, but it could also act as an accidental secondary seed disperser if some of the removed diaspores are not consumed.

The relative abundance of protein-rich animals versus diaspores can influence the numbers of diaspores ants interact with and move (Heithaus et al., 2005). Very few studies have tried to relate the availability of other food sources (particularly arthropod prey) to ant-diaspore interactions (Boulay et al., 2005). Because most ant species that interact with diaspores are generalists and often omnivorous (Christianini et al., 2012), it is expected that the availability of other food sources such as arthropod prey, will affect their interactions with diaspores. During the diaspore removal experiment in this study, *Pheidole* sp.1 and *M. opaciventris* were observed moving and transporting small, dead arthropods, and the frequency of such interactions was noted. Fewer diaspores were removed when the frequency of foraging on available arthropods was high (Appendix-Figure 3.10). Although variations in availability of arthropod prey were not assessed, the claim can be made that ants will forage more on their preferred food item when different food types are available (Detrain et al., 2000). A study carried out in Atlantic forest showed that Ponerine ants showed a preference for arthropod prey compared to diaspores, hence their interactions with diaspores was affected by the availability of arthropod prey (Pizo et al., 2005). Since more diaspores were removed by ants when they forage less on arthropod prey, it is possible that ants within NNFR prefer arthropod prey to diaspores and this has been shown for *M. opaciventris* in Cameroon (Kenne and Dejean, 1999). Hence it is expected that when arthropod prey availability is high, fewer interactions between ants and diaspores will occur. This assumption and others arising from the results of this study should be tested in future work as they may

have implications for diaspore dispersal, and subsequent germination and seedling recruitment.

3.8.8 Seed cleaning; potentially beneficial ant-diaspore interaction for seeds in NNFR

By consuming fruit tissue and arils from diaspores, ants "clean" diaspores; a behaviour that can be beneficial to plants because cleaned diaspores have been shown to be less vulnerable to pathogenic attack and have improved germination success (Ohkawara and Akino, 2005, Oliveira et al., 1995). Although only four of 13 diaspores benefitted from on the spot seed cleaning during this study, this may be a more beneficial interaction for diaspores in NNFR given the very short dispersal distances and the uncertainty especially, of the status of *Pheidole* with respect to predation. Hence, plant species such as the arillate *P. pinnata* that are too large to be moved (mean weight of 0.34 g) but are cleaned by ants in NNFR may benefit from ant cleaning activity which is known to lower diaspore mortality and enhance germination success (Ohkawara and Akino, 2005, Pizo and Oliveira, 2001). The effect of this seed cleaning on germination of *P. pinnata*, is investigated in chapter 5.

3.9 Conclusion

Many studies on diaspore dispersal focus on vertebrate-diaspore interactions involving large diaspores (Chapman and Russo, 2007, Forget, 1990, Jordano, 2000, Levey et al., 2002). Since many Afrotropical plants produce small diaspores (Chapman et al., 2016), understanding faunal interactions with small diaspores deserves similar attention. In this pioneer study of ant-diaspore interactions in a West African montane forest, I found that interactions were not very frequent and were limited to two ant species and speculate that this is partly due to low ant diversity within the reserve.

Diaspore size strongly influenced diaspore removal, although other diaspore traits not investigated may be involved, and ants are probably interacting with many other diaspores not investigated in this study. Ants in NNFR may also be exploiting diaspores that provide greater rewards relative to the effort expended, and the

influence of diaspore traits on removal rate by ants will be explored in the next chapter.

There was clear evidence for diaspore removal by ants, although diaspore 'dispersal' distances were small, relative to studies in the Neotropics. Thus, removed diaspores are not likely to benefit from a significant reduction in negative distance-dependent (NDD) effects as has been hypothesised for true ant-dispersed (myrmecochorous) diaspores. However, seed shadows and spatial patterns of seedling recruitment within the reserve may be altered through the removal of diaspores by ants. Removal of *T. orientalis* by *Pheidole* sp.1 is thought to be evidence of predation since seeds devoid of any fruit/fleshy reward were removed by this ant, whereas removal of *T. orientalis* by *Myrmicaria opaciventris* is hypothesised to be dispersal. However, in both cases, the data are insufficient to draw definite conclusions, and despite the assumption that *Pheidole* is a seed predator, there is some evidence that it could be playing a dual role as an accidental secondary disperser of *T. orientalis.* Based on these results, it can be said that ant-diaspore interactions in NNFR are both antagonistic and mutualistic.

The results of this study support the hypothesis that interactions between ants and diaspores are diffuse and asymmetrical (Warren and Giladi, 2014), and demonstrate that their benefits to diaspores are far from universal, but rather, are context dependent; varying with ant-plant combinations, geographical location and microhabitat conditions. Based on these findings, I suggest that interactions may likely involve the most abundant ant species, especially if they are omnivorous. Also, ant-mediated dispersal of non-myrmecochorous diaspores in NNFR and possibly other Afromontane habitats where the most abundant species are small Myrmicinae, will likely benefit small diaspores, while seed cleaning leading to reduced diaspore mortality and increased germination, may be the more beneficial interaction for non-myrmecochorous diaspores in these habitats.

3.10 Appendix

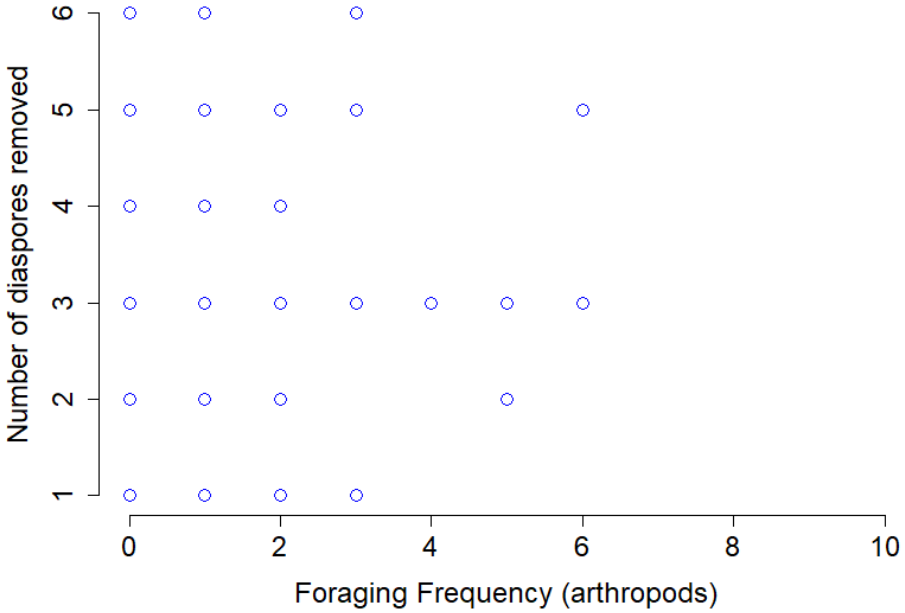

Figure 0.8: Number of diaspores removed by ants per experimental depot and foraging frequency on arthropod prey.

Chapter 4: Factors Affecting Ant-Diaspore Interactions in an Afromontane Forest-Grassland Landscape in West Africa

Abstract

In recent times attention has been given to ant interactions with diaspore not typically adapted to dispersal by ants in the tropics. This because the tropics harbour a rich diversity of diaspore types as well as a corresponding diversity and abundance of ant species. However, not much is known about factors that determine the frequency and nature of these interactions, especially in Afromontane habitats. Interactions may be opportunistic, or they could be influenced by factors pertaining to diaspore traits such as morphology and nutrient content or affected by foraging pattern of ants (*Pheidole*sp.1 and *Myrmicaria opaciventris*) or by competition by vertebrate seed removers. I investigate how these factors might affect ant diaspore interactions in an Afro-montane grassland-forest landscape. Diaspore availability was assessed for 12 months using seed traps across habitat. Morphological traits such as colour shade, hardiness and fresh weight of diaspores used for ant diaspore removal experiment were assessed. Proximate chemical analysis was also carried out on selected diaspores based on availability at the time of assessment. Diaspore removal experiment was also used to compare ant and vertebrate removal. Diaspore availability varied among habitat and site and the interaction between season and habitat affected diaspore availability. There was spatial and temporal variation in foraging activity of *M. opaciventris. Pheidole* sp.1 was not affected by season but was affected by site. Ants moved markedly smaller diaspores with wet mass ranging between 0.01g-0.25g and interacted with more diaspores with soft surface tissue those with intermediate and hard tissues while diaspore colour shade did not affect ant interactions. The impact ants and vertebrates have on the removal of diaspores from the ground in NNFR did not differ significantly. This study demonstrates that only plants that produce small diaspores within NNFR can be moved by ant. However, diaspores with soft tissues surface might be exploited by ants irrespective

of size. The seasonal difference in foraging activity of the two ants could mean that one, or both ant species interact with available diaspores all year round. The seasonal and habitat effect on diaspore availability in NNFR indicates that plants that fruit during the wet season and in the forest habitat, are important for ant-diaspore interactions within NNFR. And we may conclude that ants as well as vertebrate seed predators, are important in removing available diaspores in Ngel Nyaki Forest Reserve.

4.1 Introduction

The tropics harbour a rich diversity of diaspore types (Corlett, 1996, Howe and Smallwood, 1982, Jordano, 2000) and a correspondingly high diversity and abundance of ant species (Hölldobler and Wilson, 1990). This may partly explain why ant-diaspore interactions in the tropics involve both myrmecochorous (i.e., diaspores specifically adapted to dispersal by ants) and non-myrmecochorous diaspores (Christianini et al., 2007, Christianini et al., 2012, Passos and Oliveira, 2003, Pizo and Oliveira, 2000). Consequently, ants may also influence the regeneration of non-myrmecochorous plants (Camargo et al., 2016, Pizo and Oliveira, 2001) as they do myrmecochorous plants. Tropical ecosystems also have high diaspore productivity, with the production of fleshy fruits ranging between 180 – 1000 kg ha^{-1} (Jordano, 2000). This high biomass of different diaspores results in an abundance of non-myrmecochorous diaspores on the forest floor for ant exploitation (Hölldobler and Wilson, 1990, Jordano, 1992).

Seed dispersal in African forests relies mainly on vertebrates such as birds (Cordeiro and Howe, 2003, Holbrook and Smith, 2000) and mammals (Campos-Arceiz and Blake, 2011, Dutton et al., 2014, Feer, 1995, Gautier-Hion et al., 1993). This may explain why little attention has been given to ant-interactions with non-myrmecochorous diaspores and their contribution to dispersal in African forests. This study is the first to focus on the possible role of ants in interacting with, and dispersing non-myrmecochorous diaspores in this ecoregion. Also, not much is known about factors that determine the frequency and nature of these interactions, especially in Afromontane habitats. Interactions may be purely opportunistic, or they

could be influenced by diaspore traits such as morphology and nutrient content. Additionally, the relative abundance of alternative food sources available to ants may influence ant-diaspore interactions as has been found in Neotropical (Pizo et al., 2005) and temperate regions (Giladi, 2006). Such knowledge would be particularly valuable in understanding how ants can contribute to restoration and conservation projects in degraded tropical montane forests.

4.2 Influence of diaspore characteristics on ant-diaspore interactions

It has been well documented in other parts of the world, that diaspore characteristics such as size, mass, and the presence of an elaiosome, influence ant interactions with myrmecochorous plants (Giladi, 2006, Gomez et al., 2005, Hughes and Westoby, 1990, Hughes and Westoby, 1992). However, nothing is known about how ants interact with diaspores of non-myrmecochorous plant species in the Afrotropics (Böhning-Gaese et al., 1999, Pizo and Oliveira, 2001). Tropical montane habitats harbour smaller diaspores compared to tropical lowland forests where diaspores are on average larger (Chapman et al., 2016), and small size is a major morphological trait that strongly influences which diaspores ants interact with (Gorb and Gorb, 1995, Pizo and Oliveira, 2001). Since ants are small, they are more able and likely to move small diaspores than large ones (Kaspari, 1996, Leal and Oliveira, 1998, Levey and Byrne, 1993, Pizo and Oliveira, 2001). Larger diaspores may be exploited in other types of interactions such as diaspore cleaning (Christianini et al., 2007, Christianini et al., 2012, Oliveira et al., 1995).

The nutrient content of diaspores, such as protein, carbohydrate, lipids, and minerals per unit mass, are other important diaspore traits which influences animal choice during foraging (Jordano, 2000). In particular, it has been shown that ant interactions with both myrmecochorous and non-myrmecochorous diaspores are influenced by lipid content (Christianini et al., 2012, Passos and Oliveira, 2004, Pizo and Oliveira, 2001). In a study of ant interactions with non-myrmecochorous diaspores in Brazil, ants interacted with and removed lipid-rich diaspores significantly more frequently than lipid-poor ones (Pizo and Oliveira, 2001). However, while lipid/fat content is

the main nutrient driver of ant-diaspore interactions (Fischer et al., 2008, Pizo and Oliveira, 2001), there is some evidence that protein and carbohydrate content may also influence ant-diaspore interactions (Fischer et al., 2008). It is not known to what extent diaspores within Afromontane habitats vary in nutritional content, nor how such variations influence ant interactions and removal rates of diaspores of different plant species.

4.3 Synchrony between diaspore availability and periods of peak ant activity

Plant species whose diaspores are adapted specifically for dispersal by ants (specialised myrmecochorous plants) often fruit when ant activity is high (Giladi, 2006, Guitian and Garrido, 2006). Therefore, it might be expected that ant-diaspore interactions will be higher when ants are more active. Relating diaspore availability to periods of peak ant abundance and activity could help to identify diaspores with which ants are likely to be interacting.

4.4 Competition with vertebrate predators for diaspores

Predation of diaspores by vertebrates such as rodents and birds is a major source of mortality for small and medium-sized diaspores and has the potential to affect ant-diaspore interactions by reducing the availability of diaspores for ants. The rate at which ants can remove diaspores from the ground relative to other seed predators/dispersers influences their effectiveness as dispersers (Heithaus et al., 2005, Kerley, 1991). It is therefore important to determine whether the rate at which ants remove diaspores is affected by interference from other seed competitors such as rodents. Ants can act as both primary and secondary dispersers because they move diaspores directly from beneath parent plants, and those dropped by other vertebrates. Whether acting as primary or as secondary dispersers, they remove diaspores from predation zones to their nests, and act as rescuers of diaspores that would otherwise be consumed by vertebrate seed predators (Beattie, 1985, Christianini and Oliveira, 2009, Pizo and Oliveira, 1998).

4.5 Aim

This chapter aims to investigate some key biotic factors that might influence ant-diaspore interactions in Ngel Nyaki Forest reserve (NNFR). The objectives were to:

1. Determine the effect of diaspore availability on diaspore removal rates

2. Investigate morphological and chemical traits of selected diaspores and determine their influence on removal by ants

3. Determine the relative contributions of vertebrate seed-predators and ants to diaspore removal

The null hypothesis were:

(i) Diaspore availability will not vary among habitats and between seasons

(ii) There is no spatial and seasonal variation in the foraging activity of ants (*Myrmicaria opaciventris* and *Pheidole* sp.1) that interact with diaspores in NNFR.

(iii) Morphological traits and chemical characteristics of diaspores does not influence ant-diaspore interactions

(iv) The relative activity of small vertebrate seed predators will not affect ant-diaspore interactions, and this varies between seasons.

4.6 Methods

4.6.1 Study site

The study was carried out in three sites within NNFR each comprising grassland, forest edge and forest habitats (detailed description in Chapters 1 and 2).

4.6.2 Assessment of spatial and temporal variation in diaspore availability

Seed traps were used to assess the abundance of diaspores across seasons and habitats. Seed traps were made from a 0.5 x 0.5 cm piece of mesh netting (mesh size 0.6 x 0.6 mm) held in place with a wooden frame 30 cm above the ground (Barnes and Chapman, 2014). Traps (78 in total) were placed at 13 points on six established transects (see Chapter 2) along forest to grassland gradient starting from the forest edge (0 m from the outermost forest canopy) and going in opposite directions into forest and grassland at 5 m, 10 m, 20 m, 40 m, 80 m and 160 m after the method of (Barnes, 2011, Kotze and Samways, 1999, Kotze and Samways, 2001).

Traps were monitored weekly for 12 months, and all seeds and fruits (collectively, diaspores) found in each trap were collected, counted and identified to the lowest taxonomic level possible. Diaspores were weighed and sorted by size; seeds < 1g were categorised as small and >1g as large, because diaspores weighing 1g and below, are categorized as small diaspore is range that ants might be able to move. Diaspores weighing greater than 1g are usually too large for ants to move (Pizo and Oliveira, 2001).

4.6.3 Measurement of diaspore morphological traits

To determine if morphological traits influence how ants interact with diaspores, 20 ripe diaspores were collected from at least five individual trees of 13 plant species used in the diaspore removal experiment in Chapter 3. They included: *Allophylus africanus, Bridelia speciosa, Celtis gomphophylla, Antidesma* sp., *Harungana madagascariensis, Macaranga occidentalis, Paullinia pinnata, Polyscias fulva, Psorospermun aurantiacum, Psychotria* sp., *Rauvolfia vomitoria, Trema orientalis* and *Zanthoxylum leprieurii*.

Morphological traits that were assessed in addition to diaspore weight were: i) hardness, and ii) colour shade (light, intermediate or dark). Hardness of diaspores was assessed as i) 'soft', when diaspores could easily be squashed between fingers, ii) 'medium', when diaspores could easily be marked with fingers but not easily squashed, and iii) 'hard', when diaspores could not be marked with pressure from

fingers or squashed (Roselli, 2014). A biological trait, mode of dispersal (whether bird or mammal-dispersed) was included. This information was obtained from unpublished data collected over the last six years on seed dispersal mechanisms within NNFR.

4.6.4 Chemical characteristics of diaspores

Chemical traits of diaspores were evaluated to investigate if certain chemical traits attracted ants. Ripe diaspores of five plant species, three with which ants had been observed interacting (from opportunistic observations and diaspore removal experiment described in Chapter 3), and two which ants had not been observed interacting with. These include: fruits and seeds of *T. orientalis* and *P. pinnata*, and seeds of *C. gomphopyla* (species with which ants interacted), and *R. vomitoria* and *P. aurantiacum* were analysed. They were collected and sent to the Biochemistry Department of the National Institute for Veterinary Research, Vom, Nigeria for chemical analysis. Selected diaspores were all less than 1g, well within the range of weights which ants would be able to move (Pizo and Oliveira, 2001). Selection of diaspores for the analysis was based on their availability at the time of the study.

Seven chemical traits were analysed: moisture content, crude fibre, crude protein, crude fat, nitrogen-free extractive (carbohydrate content), calcium and phosphorus. The analysis was carried out using the proximate analytical methods described in Dublecz (2011).

4.6.5 Measurement of *Myrmicaria opaciventris* and *Pheidole* sp.1 foraging activity

Foraging activity of *M. opaciventris* and *Pheidole* sp.1 was assessed using tuna baits along each transect within the three different study sites every month from June 2016- May 2017, encompassing both wet and dry seasons. Tuna baits (20 g) were placed on filter paper at 13 stations laid out in the same pattern as the seed traps on each transect (detailed description in chapter 2) along the grassland to forest gradient between 0900 hrs and 0010 hours. Baits were checked after 30 and 60 minutes, and the number of ants of each species feeding at each station was recorded (Beattie and Culver, 1981, Perfecto and Vandermeer, 1996). Activity of *M. opaciventris* and

Pheidole sp.1 was described as the number of baits each species visited, divided by the total number of bait stations (Beattie and Culver, 1981)

4.6.6 Diaspore removal experiment - ants versus vertebrates

This experiment compared diaspore removal rates between ants and vertebrate predators such as mice and birds which are likely to prey on small diaspores. Removal depots were set beneath five individuals of *T. orientalis* trees to assess removal by ants and vertebrates within the tree's natural environment. The trees were separated by a distance of at least 1 km. The removal rate of vertebrate and ants was assessed using experimental diaspore depots. Experimental depots comprised of an open "non-vertebrate excluded" (open) depot (Fig 4.1) where other predators such as rodents and birds were allowed access to the diaspores (Christianini et al., 2007, Christianini and Oliveira, 2010) and an enclosed "vertebrate excluded" (closed) depot which consisted of fruits placed in an inverted plastic pottle (Fig 4.2) that had 1cm by 8cm holes cut at ground level for accessibility to ants but which excluded vertebrates (Roselli, 2014, Vaz Ferreira et al., 2011).

Figure 0.1: Non-vertebrate excluded (open) depot

Figure 0.2: Vertebrate excluded (closed) depot

Depots (open and closed) were placed 2 m apart along 20 m transects at 2, 5, 10, and 20 m from the base of the trunk of the five *T. orientalis* focal trees (Pizo and Oliveira, 1998). Experiments were carried out monthly from August 2016 to July 2017 and set-up between 0900 hrs and 1100 hrs. Depots were checked after 24 hours, and the number of fruits removed from each depot was recorded. During monthly repeats of the experiment, the position of open and enclosed depots along transects was alternated to ensure that visits by ants and other vertebrates to both treatments were not influenced by the memory of previous visits.

4.7 Data analysis

Data were analysed using R version 3.2.4 with the *lme4* and *car* packages. For every test, p-values ≤ 0.05 were considered significant. ANOVA was used for model comparison and for removing factors that had the least significant effect. Akaike Information Criteria (AIC) were used to select the best fit model (Crawley, 2002a, Symonds and Moussalli, 2011). Models with the fewest factors were selected as the best fit models when models were not significantly different. The *blmeco* package in R was used to check the best fit models for overdispersion occurring because of clustering of the response variable. An observational-level random effect was included in models to account for overdispersion (Harrison, 2014).

4.7.1 Diaspore availability

A generalised linear mixed-effect model (GLMM) with binomial distribution was used to assess variation in diaspore availability between two seasons (wet and dry) and across the different habitats (grassland, edge, and forest) and sites. The presence or absence of diaspores was the response variable, while the independent (fixed effect) variables were 'habitat' and 'site' and 'season' and their interactions. 'Transect', 'trap position' and 'trap ID' were included as random effects to correct for pseudo-replication (Crawley, 2002b).

Relative abundance of diaspores was assessed as the number of diaspores of the different plant species collected in a trap. A GLMM with Poisson distribution was used to assess variation in diaspore abundance between two seasons (wet and dry) and across the different habitats (grassland, edge, and forest). The 'number of diaspores' was the response variable, while the independent (fixed effect) variables were 'habitat', 'season', 'site', 'plant species', and their interactions. 'Transect', 'trap position' and 'trap ID' were included as random effects to correct for pseudo-replication (Crawley, 2002b).

Diaspore density was calculated using the formula:

$$Diaspore\ density = \frac{\sum Tn}{Nt}$$

Where Tn = Actual number of diaspores collected per trap, Nt = total area surveyed.

4.7.2 Diaspore morphological characteristics

An independent t-test was used to compare the weight of diaspores moved by ants with those that ants did not move. Colour shade and hardness of diaspores that ants interacted with (moved and cleaned on the spot) and those that ants did not interact with during diaspore removal and seed cleaning experiments were compared using a Chi-square test.

4.7.3 Chemical properties of diaspores

A principal component analysis (PCA) based on correlation matrix was used to assess the similarities and dissimilarities of chemical traits among the different diaspores. The component loading was used to describe the chemical similarities, dissimilarities, and patterns.

4.7.4 Foraging activity of *Pheidole* sp.1 and *Myrmicaria opaciventris*

A one-way analysis of variance (ANOVA) was used to assess how ant foraging activity varied between seasons (wet and dry) and across the three study sites. Season comprised of grouped monthly data for August-October 2016 and May-July 2017 (rainy/wet season) while the dry season included the months from November 2016 to March 2017.

4.7.5 Vertebrate and ant diaspore removal

Vertebrate and ant diaspore removal were compared using a GLMM with Poisson distribution. The 'number of diaspores removed' was the response variable, while the independent (fixed effect) variables were, 'season', 'enclosure treatment', 'habitat,' and the interactions between these factors. 'Depot position' and 'tree ID' were included as random effects to correct for pseudo-replication (Crawley, 2002b).

4.8 Results

4.8.1 Diaspore availability

In total, 1091 diaspores (fruits and seeds) distributed across 20 plant families and 32 species were collected from the seed traps (Table 4.1).

Table 0.1: Species of fruits and seeds sampled using seed traps in the edge, forest and grassland habitats in Ngel Nyaki Forest Reserve.

Family	Species	Edge	Forest	Grassland	Diaspore size
Achariaceae	*Dasylepis racemose*	0	1	0	large
Annonaceae	*Isolona dieghtonii*	1	0	0	large
Apocynaceae	*Landolphia* sp.	5	7	0	large
	Rauvolfia vomitora	9	0	0	small
Araliaceae	*Polyscias fulva*	9	0	0	small
Clusiaceae	*Garcinia smeathmanii*	1	0	0	large
Compositae	*Vernonia* sp.	5	0	10	small
Euphorbiaceae	*Bridelia speciosa*	83	23	7	small
	Macaranga monandra	7	53	0	small
Fabaceae	*Acacia* sp.	2	13	0	small
	Anthonotha noldae	1	0	0	large
Leguminosae	*Unidentified* sp.1	143	53	22	large
	Lobelia sp.	4	0	0	small
	Newtonia buchananii	2	25	0	large
	Parkia filiocoidea	0	1	0	large
Guttiferae	*Psorospermun aurantiacum*	133	4	21	small
Lauraceae	*Beilschmiedia mannii*	18	2	0	large
Meliaceae	*Carapa grandiflora*	0	2	0	large
	Entandrophragma angolense	0	1	0	large
Moraceae	*Ficus* sp.	29	15	0	large
Oleaceae	*Chionanthus africanus*	4	0	0	large
Rubiaceae	*Oxyanthus* sp.	0	1	0	large
	Psychotria sp.	137	36	7	small
	Unidentified sp.2	19	24	0	small
Rutiaceae	*Clausena anisata*	0	5	0	small
Santalaceae	Mistletoe	0	1	4	small
Sapindaceae	*Paullinia pinnata*	65	34	0	small
Sapotaceae	*Pouteria altissima*.	0	0	1	large
Ulmaceae	*Celtis gomphophylla*	1	0	0	small
Poaecae	Unidentified Grass	0	0	4	small
Unidentified	Unidentified sp. 3	2	0	0	small
	Unidentified sp. 4	28	6	0	small
	Total	**708**	**307**	**76**	

Of the total, 708 (mean abundance per trap =7.4) diaspores were collected from the edge habitat, while 307(mean abundance per trap =12.8) and 76 (mean abundance per trap =1.6) diaspores were collected from the forest and the grassland habitats respectively. Seventeen (53%) of the 32 species present in the diaspore samples had small diaspores (weight < 1g), while 15 (47%) had large diaspores (weight > 1 g). Overall diaspore density was calculated to be 0.29 m^2.

4.8.2 Spatial and temporal variation in diaspore availability

Diaspore availability varied among habitats and sites, and the analysis showed that significantly more diaspores were available in the edge habitat than in the grassland and forest habitats. Season did not have a significant effect on diaspore availability (Table 4.9) however, interactions between habitat and season (χ^2 =7.57, df = 2, p < 0.05) and between site and season (χ^2 =12.3, df = 2, p < 0.01) had significant effects on diaspore availability (Table 4.2).

Table 0.2: Results of the generalised linear mixed model with binomial distribution showing the effect of habitat, site, season, and their interactions on the number of diaspores available. Significance is denoted by asteriks at p = 0.05

Source of variation	**Chisq**	**df**	**p-value**
Habitat	50.1	2	< 0.0001*
Site	26.0	2	< 0.0001*
Season	1.10	1	0.29
Habitat: Season	7.57	2	< 0.05*
Site: Season	12.3	2	< 0.01*

4.8.3 Spatial and temporal variation in the mean abundance of available diaspores

The interaction between season and habitat (Figure 4.3) affected the number of available diaspores significantly ($p < 0.0001$). The wet season had a significant positive effect on the number of diaspores available in the forest habitat ($\beta = 0.84, \pm 0.24$ (SE), $p < 0.001$) and a significant negative effect on the number of diaspores available in the grassland habitat ($\beta = -0.79, \pm 0.23$ (SE), $p < 0.01$) (Appendix 4.1). The interaction between season and plant species also had a significant effect on the number of available diaspores. (Table 4.3). Species availability was affected either positively or negatively by season (details in table 4.10 in the appendix).

Table 0.3: Results of the generalised linear mixed model with Poisson distribution showing the effect of habitat, site, season, month and their interactions on the number of available diaspores. Significance at $p < 0.05$ is denoted by an asterisk

Sources of variation	**Chisq**	**df**	**p-value**
Habitat	0.77	2	0.68
Site	2.67	2	0.26
Season	2.11	1	0.14
Species	79.7	33	<0.0001*
Species: Season	54.3	9	< 0.0001*
Habitat: Season	18.9	2	< 0.001*

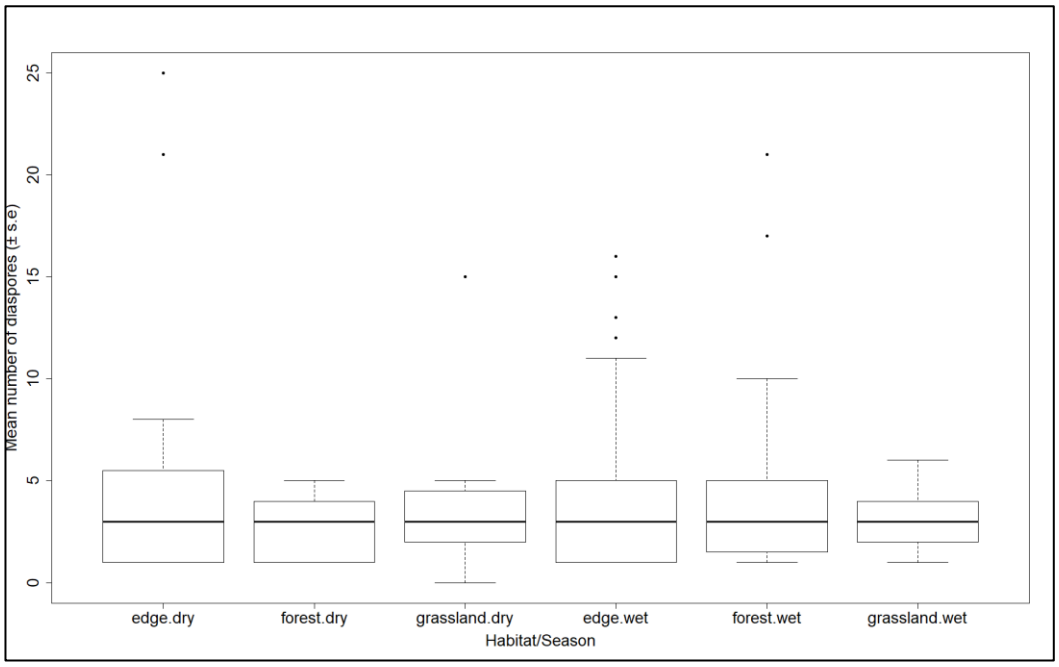

Figure 0.3: Effect of seasonal and habitat on mean diaspore numbers per trap. Number of traps: edge dry season = 35, forest dry season = 10, grassland dry season = 20, edge wet season = 42, forest wet season = 12, grassland dry season = 24.

4.8.4 Effect of season and site on foraging activity of *Pheidole* sp.1 and *Myrmicaria opaciventris*

Foraging activity of *M. opaciventris* differed significantly between seasons ($F = 4.37$, $df = 1$, $p < 0.05$) and sites ($F = 11.42$, $df = 2$, p-value < 0.0001). This species showed higher mean foraging activity in the dry season (0.57± 0.3 s. e) compared to the wet season (0.42 ± 0.42 s. e) (Figure 4.4), and in Site A compared with sites B and C (Figure 4.5)

Foraging activity of *Pheidole* sp.1 was not affected by season ($F = 1.41$, $df = 1$, $p = 0.23$) but differed significantly among the three study sites ($F = 10.39$, $df = 2$, $p < 0.001$). This species showed lower mean foraging activity in Site A relative to sites B and C (Figure 4.5).

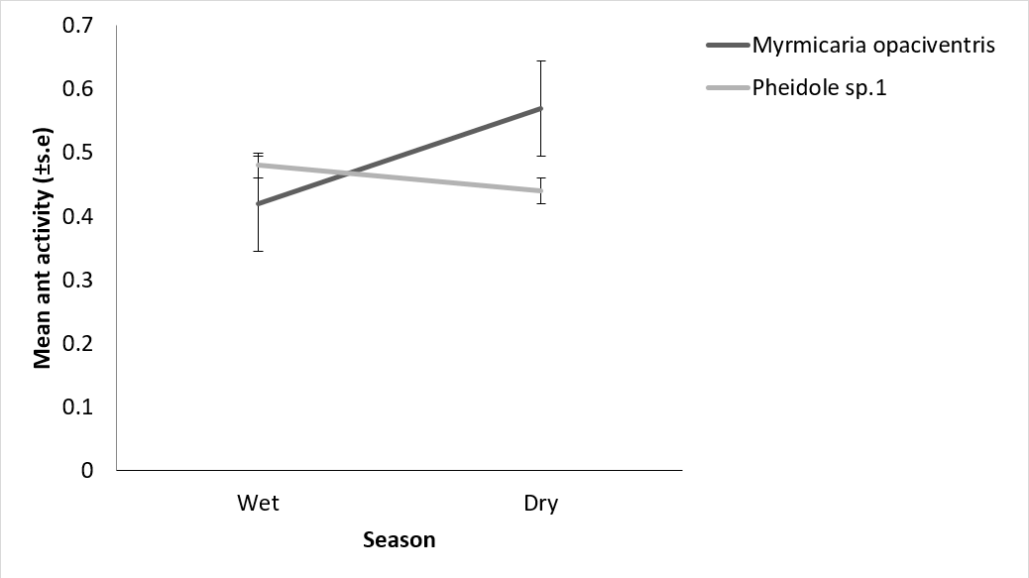

Figure 0.4: Effect of season on foraging activity of *Myrmicaria opaciventris* and *Pheidole* sp.1 in Ngel Nyaki Forest reserve from the dry season of 2015 to the wet season of 2016. Foraging activity: assessed as ant attendance to baits (Tuna) by the total number of baits.

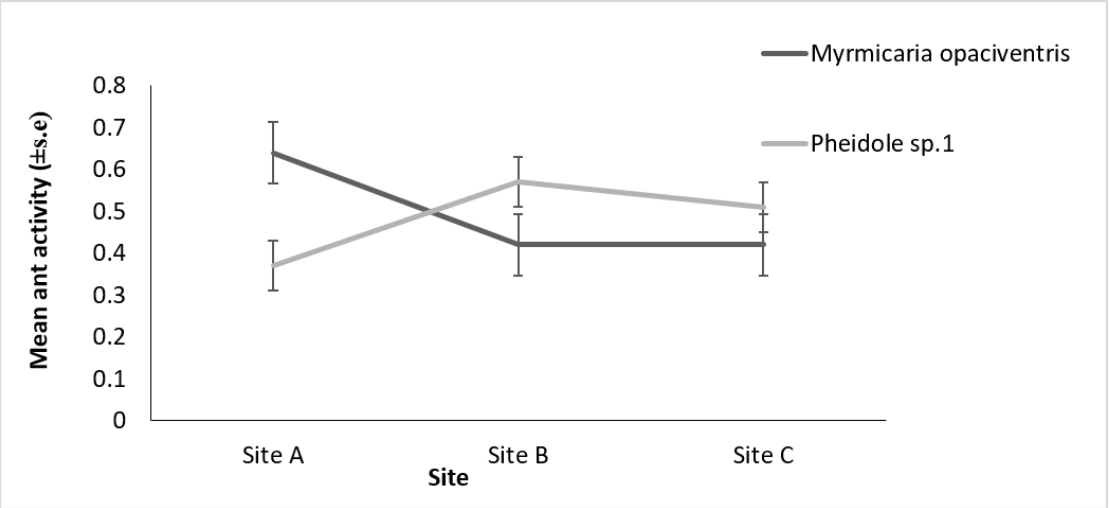

Figure 0.5: Mean foraging activity (averaged over 11 months) of *Myrmicaria opaciventris* and *Pheidole* sp.1 across three study sites in Ngel Nyaki Forest Reserve. Foraging activity: assessed as ant attendance to baits (Tuna) by the total number of baits.

4.8.5 Diaspore morphological characteristics

Twenty-two diaspore types (fruits and seeds) from 12 plant species (Table 4.4) were used in the diaspore removal experiment (details in Chapter 3). When compared, the mean weight of diaspores that were moved by ants differed significantly from that of diaspores which ants did not move ($t = 3.54$, $df = 14.8$, $p < 0.01$), although there was more variation in size of diaspores that ants did not move (Figure 4.6). The mean weight of diaspores moved by ants was markedly smaller (0.03 ± 0.01 s. e; range 0.01 – 0.25, than that of diaspores not removed by ants (0.23 ± 0.06 s. e; range 0.05 – 0.6).

Colour shade of diaspores (dark, intermediate or light) did not influence interaction by ants significantly ($\chi^2 = 0.34$, $df = 2$, $p = 0.84$). Hardness of diaspores (hard, medium or soft) strongly affected ant interactions ($\chi^2 = 84.4$, $df = 2$, $p < 0.0001$). Ants interacted more frequently with diaspores which had soft tissue than hard and medium diaspores (Figure 4.7).

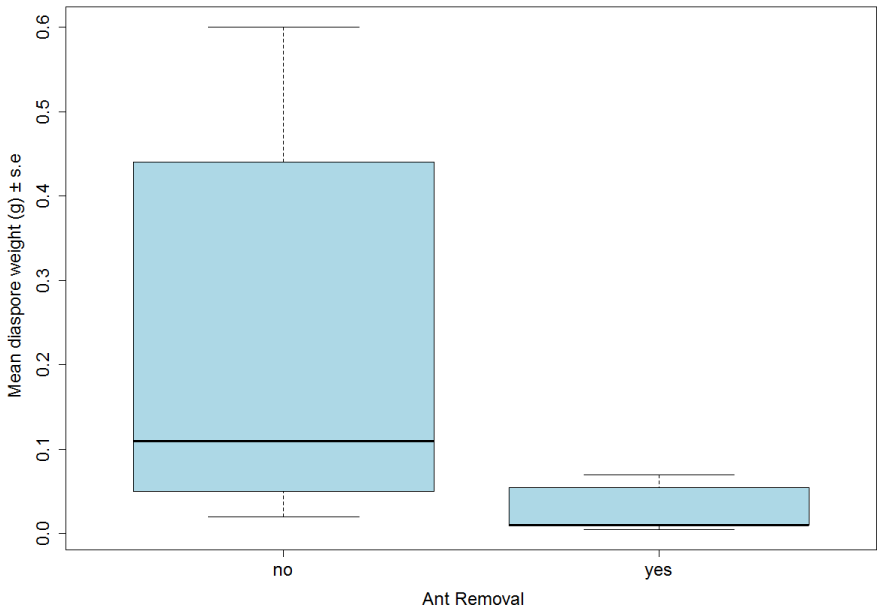

Figure 0.6: Mean weight of diaspores moved vs diaspores not moved by ants. "Diaspores not moved" = no, "diaspores moved" = yes.

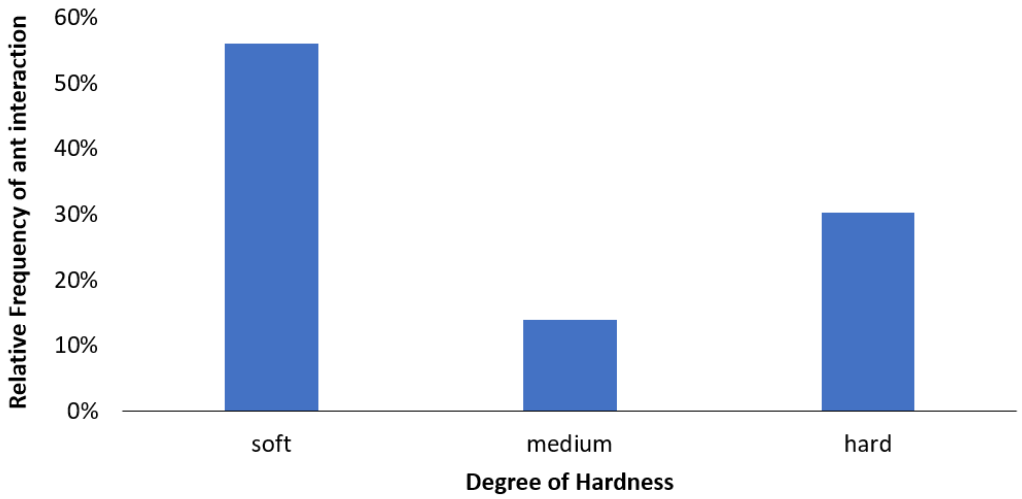

Figure 0.7: Ant response to the degree of diaspore hardness. Soft = easily squashed with the finger, medium = easily be marked with the finger but not easily squashed, hard = not easily marked with pressure from fingers or squashed.

Table 0.4: Morphological traits of diaspores used for diaspore removal experiments. Diaspores moved in red. Colour shades are indicated by † = light, ‡ = intermediate and ⁑ = dark. Diaspore type: F = fruit, S= Seed, C=cleaned seed (*Trema orientalis*)

Plant species	Diaspore Type	Fresh Weight (g)	Ant Interaction	Colour	Hardness	Dispersal syndrome
Allophylus africanus	F	0.60	None	Orange †	Soft	Bird/Mammal
	S	0.07	None		Soft	
*Bridelia speciosa**	F	-	None	Green ‡	Medium	Bird/Mammal
	S	0.07	Moved	Brown ‡	Medium	
Celtis gomphophylla	F	0.21	None	Yellow †	Soft	Bird/Mammal
	S	0.01	Moved	Brown ‡	Medium	
Antidesma sp.	F	0.11	None	Light green†	Hard	Mammal
	S	0.02	None	Yellow †	Hard	
*Harungana madagascariensis**	F	-	Moved	Red †	Soft	Bird
	S	-	Moved	Black ⁑	Medium	
Macaranga occidentalis	F	0.04	None	Black ⁑	Soft	Bird
	S	0.03	None	Black ⁑	Hard	
Paullinia pinnata (arillate)	F	0.53	Cleaned	White/black†	Soft	Mammal
	S	0.34	None	Brown/black‡	Hard	
Polyscias fulva	F	0.07	Moved	Purple/black⁑	Soft	Bird/Mammal
	S	0.01	Moved	Light-brown†	Soft	
Psorospermun aurantiacum	F	0.56	Cleaned	Red †	Soft	Bird
	S	0.05	None	Brown ‡	Soft	
Psychotria sp.	F	0.23	None	Red ‡	Soft	Bird
	S	0.05	None	Orange †	Medium	
Rauvolfia vomitoria	F	0.56	None	Orange †	Soft	Bird
	S	0.09	None	Brown ‡	Medium	
Trema orientalis	C	0.005	Moved	White †	Hard	Bird
	F	0.04	Moved	Black ⁑	Hard	
	S	0.01	Moved	Brown ‡	Hard	
Zanthoxylum leprieurii	S	0.25	Moved	Brown ‡	Medium	Bird

*Diaspores of *B. speciosa* and *H. madagascariensis* were not weighed (experiments conducted through field assistants).

4.8.6 Chemical characteristics of selected diaspores based on proximate analysis

Chemical traits (moisture content, crude fibre, crude protein, crude fat, nitrogen-free extractive (carbohydrate content), calcium and phosphorus content) were measured for the six plant species whose diaspores were moved by ants in the diaspore removal experiments in Chapter 3, and the one plant species (*P. pinnata*) that was cleaned on the spot (Table 4.4).

The first three dimensions of the PCA together, explained 84% of the measured variation in chemical characteristics among the diaspore types. These dimensions respectively accounted for 40%, 25% and 19.5% of the variance in chemical traits. Five variables (calcium, crude fat, moisture, crude protein, and ash content), which had the longest vectors in the PCA matrix, accounted for the variations in the first three dimensions of the PCA (Figure 4.8).

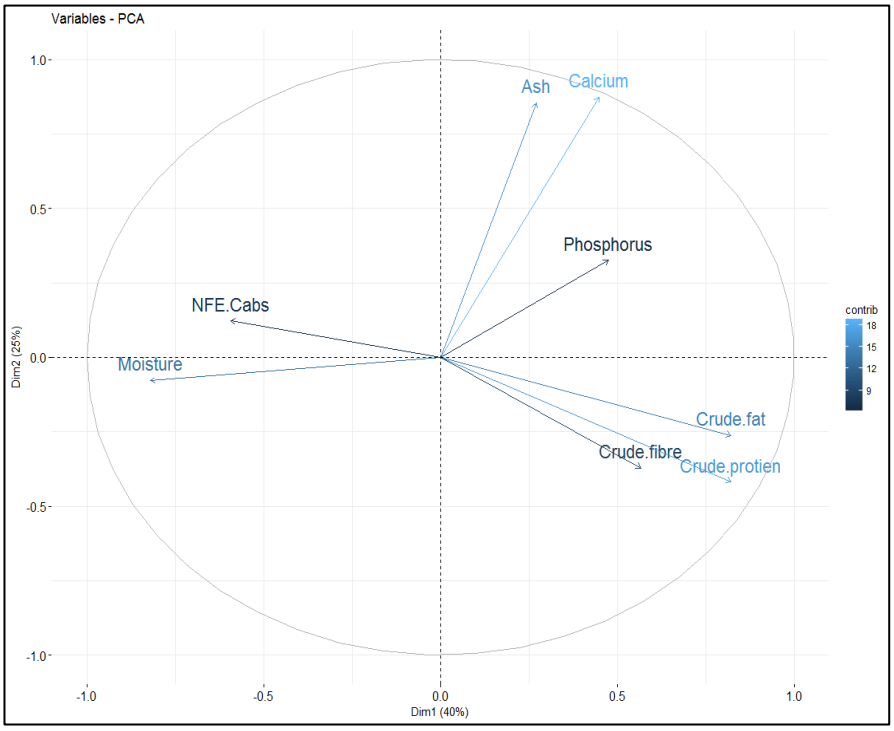

Figure 0.8: Correlation circle showing the optimal representation of variables and the strength of their contribution to dimension 1 and 2 of PCA for diaspore chemical characteristics. Longer and lighter arrows show variable vectors that contribute more to variation.

The first dimension of variability, which accounted for 40% of the total variation, was strongly correlated with crude protein, crude fat and moisture content, showing that diaspores with high crude protein and fat, had low moisture content. The second dimension (25% variation) correlated strongly with ash and calcium content, while the third dimension (19.5% variation) was strongly correlated to phosphorus, crude fibre, carbohydrate (NFE), moisture, and crude fat content (Table 4.5).

Table 0.5: Summary of Principal Component Analysis performed proximate analysis of diaspores showing the coefficient of each chemical content and variance explained by each dimension. Asterisks denote significant correlation coefficients.

Variable	**Dimension 1**	**Dimension 2**	**Dimension 3**
Moisture content	-0.82*	-0.07	-0.51*
Crude protein content	0.82*	-0.41	0.16
Crude Fibre content	0.56	-0.37	0.59*
Crude Fat content	0.82*	-0.26	-0.44*
Ash content	0.27	0.85*	0.21
NFE. Carbohydrate content	-0.59	0.12	0.56*
Calcium content	0.44	0.87*	0.08
Phosphorus content	0.47	0.32	-0.59*
Percentage variance	39.96%	25%	19.5%

NFE: Nitrogen free extract, a component of carbohydrate content.

Seed aril of *P. pinnata* and fruit of *T.* orientalis, contributed significantly to the variance in dimension 1 with the most extreme coefficient estimates of -3.70 and 1.78 respectively. This is illustrated by the distance separating them in the PCA

biplot (Figure 4.9). Figure 4.10 also shows that calcium, moisture, crude fat, crude protein, and ash contents, were the traits that accounted for most of the variance among diaspores.

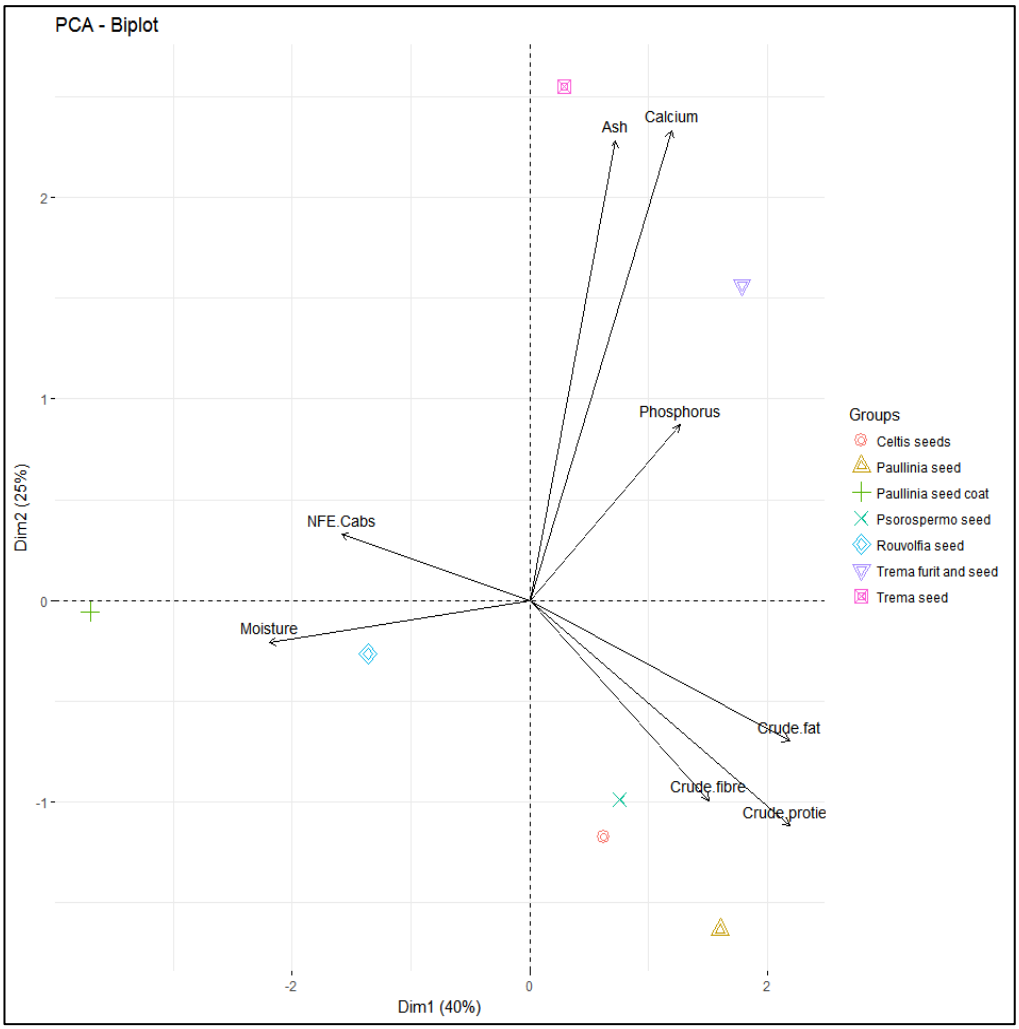

Figure 0.9: Biplot of diaspores and their chemical characteristics. The closer the diaspore type is to the variable vector on the orthogonal biplot, the stronger the association between them. The farther they are, the weaker the association.

P. pinnata seeds, *T. orientalis* fruits, and *T. orientalis* seeds, contributed significantly to the variance in the second dimension, with coefficient estimates of -1.63, 1.56 and 2.54 respectively, while *R. vomitoria* seeds, *P. aurantiacum* seeds, and *P. pinnata*

seed aril contributed the most to the variance in the third dimension with coefficient estimates of 1.90, -1.54 and -1.26 respectively (Table 4.6).

Table 0.6: Contributions of plant species/diaspore to variations in chemical composition in dimensions 1, 2 and 3 and their PCA coefficients. Asterisks denote diaspore types with the most contribution.

Diaspore type	**Dimension 1 Estimates**	**Dimension 2 Estimates**	**Dimension 3 Estimates**
Celtis gomphophylla seeds	0.62	-1.17	0.91
Paullinia pinnata seeds	1.60	-1.63*	0.29
Paullinia pinnata seed aril	-3.70*	-0.05	-1.26*
Psorospermum aurantiacum seeds	0.76	-0.98	-1.54*
Rauvolfia vomitoria seeds	-1.36	-0.26	1.90*
Trema orientalis fruit	1.78*	1.56*	-0.24
Trema orientalis seeds	0.28	2.54*	0.93

In the first dimension of the PCA, *P. pinnata* seed aril and *R. vomitoria* seeds had higher moisture content relative to crude protein and crude fat content. This is shown by their negative coefficient estimates, closer proximity to moisture content, and large distance to crude fat and protein content in the PCA biplot (Figure 4.10).

Diaspores that ants interacted with, were closer to ash, calcium and carbohydrate content on the orthogonal space, with variability significantly explained in the second dimension of the PCA (coefficient estimate of 0.72). They were further away

from moisture, crude fat and protein content, with variability significantly explained by the first dimension (coefficient estimate of -0.25) (Figure 4.10).

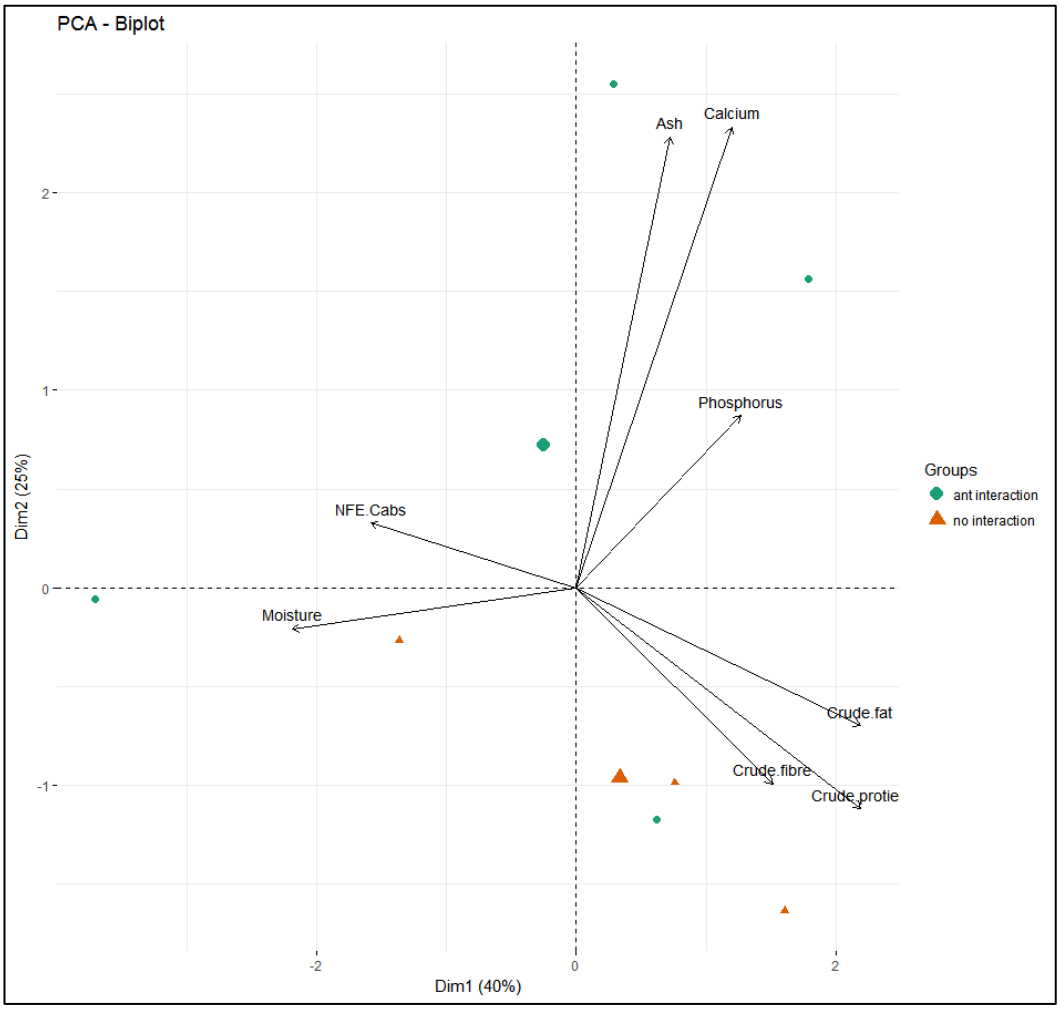

Figure 0.10: Biplot showing ant response to diaspores and their chemical characteristics (Proximate). Length of arrows shows the strength of contribution of each trait/vector. Vectors pointing in the same direction are similar, and different to vectors pointing in different directions, and symbol = scores.

4.8.7 Vertebrate and ant removal of *Trema orientalis* diaspores

The number of *T. orientalis* diaspores removed did not differ significantly between open (vertebrate) and closed (ants-only accessible) treatments (Table 4.7). Mean number of diaspores removed from the open depots was 5.02 ± 0.23 (SE) and only slightly higher than the mean number removed from the enclosed depots; 4.87 ± 0.22 (SE).

Table 0.7: Results of the generalised linear mixed model with Poisson distribution showing the effect of season, enclosure treatment, and their interactions on removal of *Trema orientalis* diaspores. Significance is denoted by an asterisk at $p < 0.05$

Source of variation	**Chisq**	**Df**	**p-value**
Season	2.29	1	0.12
Enclosure treatment	1.38	1	0.23
Season: Enclosure treatment	7.75	1	< 0.01*

The interaction between season and enclosure treatment had a significant effect on the number of diaspores removed ($\chi = 7.5$, df = 1, $p < 0.01$) (Table 4.7). More diaspores were removed from open depots than from closed depots during the wet season. However, diaspore removal between open and enclosed depots did not differ during the dry season (Figure 4.11).

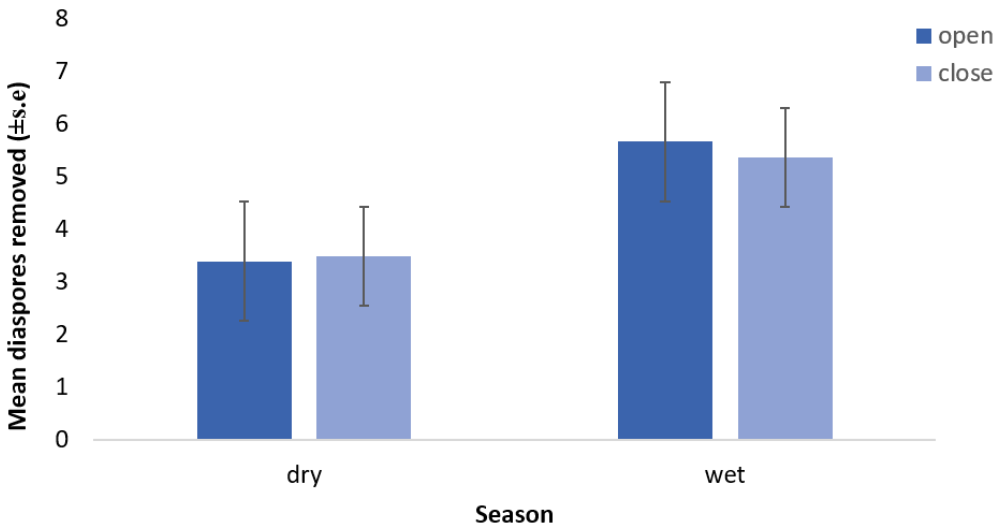

Figure 0.71: Mean number of *Trema orientalis* diaspores removed from the open and closed enclosure treatments.

4.8.8 Effect of habitat on vertebrate and ant removal of *Trema orientalis* diaspores

Habitat and enclosure treatment had no significant effect on the number of diaspores removed, but their interaction did ($\chi = 6.73$, df = 2, $p < 0.05$) (Table 4.8). More diaspores were removed from open than closed depots in the edge habitat. In contrast, more diaspores were removed from closed than open depots in the forest and grassland habitats (Figure 4.12).

Table 0.8: Results of the generalised linear mixed model with Poisson distribution showing the effect of habitat, enclosure treatment and their interactions on diaspore removal of *Trema orientalis*. Significance is denoted by asterisks at $p < 0.05$

Sources of variation	Chisq	Df	p-value
Habitat	0.06	2	0.96
Enclosure treatment	0.64	1	0.42
Habitat: Enclosure treatment	6.73	2	< 0.05*

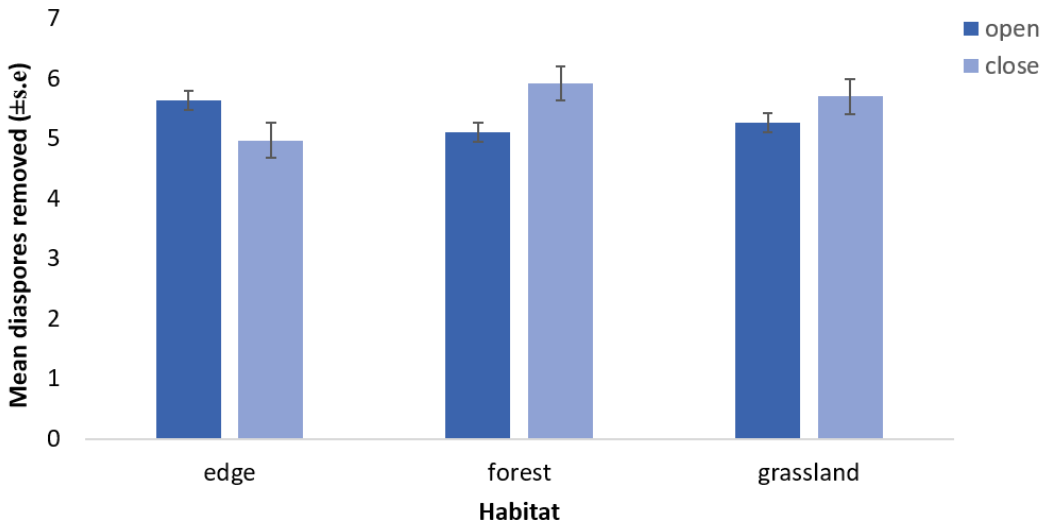

Figure 0.12: Removal of *Trema orientalis* diaspores in the open and closed enclosure treatments in the edge, forest and grassland habitats. Light blue = open depot; dark blue = closed depot.

4.9 Discussion

4.9.1 Diaspore availability and the potential for ant-diaspore interactions

African tropical forests are shaped by distinct wet and dry seasons, which influence flowering and fruit production (van Schaik et al., 1993) and a large proportion of plants in tropical forests bear animal-dispersed diaspores (Jordano, 2000) that ripen during the wet season to coincide with the peak of animal activity (Howe and Smallwood, 1982). Hence, interactions between diaspores and animals, for example, seed dispersal, are likely to occur more frequently in the wet season when fruit production is higher.

The results of this study show that diaspore availability in NNFR varies with habitat (Table 4.2) and diaspore abundance with season (Table 4.3); with more diaspores available in the forest habitat during the wet season. This finding is consistent with the fact that tropical forests show seasonal variations in diaspore availability (van Schaik et al., 1993) and can be explained by the distinct wet (April to October) and dry (November to March) seasons that characterise NNFR (Dutton and Chapman, 2014). Also, most of the diaspores of trees and shrubs within NNFR are dispersed by forest-dwelling animals, which would include chimpanzees (Dutton et al., 2014), monkeys and rodents (Aliyu et al., 2014), and birds (Roselli, 2014). This may also explain why more diaspores were more available during the wet than the dry season, particularly in the forest habitat.

The low availability of diaspores in the grassland habitat compared to the edge and the forest habitat was expected, due to the lower density of woody plant species in the grassland habitat compared to the edge and the forest habitats (Appendix- Table 4.9). Habitats with higher tree density are expected to have more diaspores than grassland habitats where few trees occur (García et al., 2011). Although the effect of diaspore availability on ant-diaspore interactions was not assessed, I speculate that diaspore availability will have a positive influence on diaspore removal by ants.

Thus, it may be inferred from the higher abundance of diaspores found in the forest, that there would be a higher frequency of ant-diaspore interactions in this habitat.

The collection of diaspores in seed traps placed within the grassland habitat is indicative of dispersal by birds which drop seeds in flight or from tree perches. This is supported by the fact that the two most abundant diaspores found in the grassland belonged to *Psychotria* sp. and *B. speciosa* -the same species with the highest number of seedlings under focal trees in the study conducted by Roselli 2014. One these species (*B. speciosa*) is among the diaspores moved by ants in Chapter 3 of this thesis. This link is important because it suggests that these species are likely to dominate the secondary forest regenerating within the grassland habitat in NNFR.

4.9.2 Foraging activity of *Pheidole* sp.1 and *Myrmicaria opaciventris*

Insect activity has been shown to vary along moisture gradients (Tauber et al., 1998), hence in highly seasonal environments like NNFR, it is expected that insect activity would vary between the wet and dry season. Contrary to this expectation, foraging activity of *Pheidole* sp.1 did not differ significantly between seasons.

This contrasts with studies in Southern Africa in which ant activity increased from the dry to the wet season (Kaspari and Weiser, 2000). Temperature and humidity are important factors that predict ant activity. High temperature and low humidity increase the risk of desiccation (Azcárate et al., 2007, Human et al., 1998, Kaspari and Weiser, 2000). Hence, ants tend to avoid desiccation by foraging in moist and cooler habitats. This is more obvious in dry ecosystems such as deserts, where ants forage at times of the day when the temperature is lower, and moisture is higher (Lighton and Feener Jr, 1989). The absence or lack of variation in foraging activity of *Pheidole* sp.1 might be an attribute of lower desiccation risk within NNFR. Although highly seasonal with wet season between April to October and a dry season from November to March, there is not much variation in the mean minimum and maximum temperature between the dry and wet season. Mean minimum and maximum temperature for the wet season in NNFR are 13 °C and 26 °C for the wet season, and 16 °C and 23 °C during the dry season (Beavon and Chapman, 2011). Hence foraging activity of ants within NNFR would likely not be affected markedly

by seasonal variations in temperature but might be affected by other factors such as food availability and microhabitat conditions.

***Myrmicaria opaciventris*:** Foraging activity for *M. opaciventris* differed significantly between the wet and dry season. There was a 10% decrease in foraging activity of *M. opaciventris* from the dry to wet season. This is consistent with the findings of a study on *M. opaciventris* carried out in lowland Cameroon that found seasonal variations in its foraging activity. Foraging activity of *M. opaciventris* was observed to be diurnal during the dry season but followed a nychthemeral cycle (night and the day) during the wet season (Kenne and Dejean, 1999). The fact that ant foraging activity was assessed only during the day may explain why higher foraging activity was recorded for *M.opaciventris* during the dry season. This species has been shown to forage exclusively during the day in the dry season, and foraging may take place either during the day or night or both (in a nychthemeral rhythm) in the wet season, (Kenne and Dejean, 1999, Kenne and Dejean, 1997).

Site had a significant effect on foraging activity of both *Pheidole* sp.1 and *M. opaciventris.* Foraging activity of *M. opaciventris* was higher for site A than the two other sites (B and C) and lower for *Pheidole* sp. 1 even though the three study sites (A, B and C) appeared to be similar in structure. Although microhabitat differences between these sites were not measured while assessing ant foraging activity, the one apparent structural difference between the sites was the slope. This was assessed in chapter 2, and it was observed that site A had the least slope with a more uniform topography in comparison to sites B and C. Variation in slope can alter the heterogeneity and complexity of a landscape, and habitat complexity is known to affect the movement of smaller species, with implications for foraging success (Wilkinson and Feener, 2007). This is consistent with lower foraging activity of *Pheidole* sp.1 on site A, but inconsistent with the higher foraging activity of *M. opaciventris* at the same site. *Pheidole* sp.1 is a much smaller ant (mean body size of 1.26 mm) than *M. opaciventris* (mean body size 1.96mm) and it is possible that the difference in body size contributes to the differences observed in foraging activity within the same landscape.

The number of *Pheidole* sp.1 foraging was seen to decrease with high numbers of *M. opaciventris* and vice versa (Figure 4.5). This relationship might also be an attribute of competition between the two species, where the presence of one might affect the presence and activity of the other. However, aggressive/competitive behaviour was not observed between the species (*Pheidole* sp.1 and *M. opaciventris*) during focal observations to assess ant activity and diaspore removal experiments carried out in chapter three. Both species were observed to forage irrespective of the presence of the other. However, it is possible that some unmeasured variable is responsible for this observation.

4.9.3 Diaspore morphological characteristics

Pheidole sp.1 and *M. opaciventris* were the only ants that moved diaspores in NNFR. They had a mean body size of 1.26 mm and 1.96 mm respectively. This indicates that they are small ants. They moved very small diaspores with a mean weight of 0.03g. Consequently, it is not surprising that there is not much variation in the size of diaspores they were observed to move (Figure 4.6). Body size is an important determinant of the range of diaspores that can be moved by ants. Tiny ant species are typically able to transport small seeds while not able to move and transport large seeds (Kaspari, 1996, Mayer et al., 2005, Pizo and Oliveira, 2001). Hence diaspore removal by ants within NNFR would be limited to trees that produce very small diaspores which will be moved only short distances (as shown in chapter 3), because small ants can move diaspores only short distances (Ness et al., 2004).

Colour shade of diaspores (light, intermediate or dark) did not significantly affect ant-diaspore removal within NNFR. Like most tropical forests, diaspores in NNFR are not adapted to dispersal by ants (non-myrmecochorous) but to dispersal by vertebrates (Barnes and Chapman, 2014, Hölldobler and Wilson, 1990). In this area, birds have shown a preference for brightly coloured diaspores with light colour shade rather than diaspores with dark colour shade (Barnes and Chapman, 2014). The lack of ant-diaspore preference based on colour shade suggests that colour shade might not be essential in the choice of diaspores with which ants interact. However, the colour shade of diaspores alone might not be sufficient in influencing ant-

interactions. This is because diaspore colour is also influenced by chemical composition (Galetti et al., 2011).

Ants interacted more frequently with softer diaspores than with diaspores that had hard or moderately hard (medium) surfaces. This is probably because soft tissues offer a more accessible food reward. Plants whose diaspores are specifically adapted to dispersal by ants (myrmechochores) produce diaspores with hard surfaces (specifically seeds) to minimize predation by invertebrates such as granivorous ants (Rodgerson, 1998). However these diaspores possess a food reward in the form of soft fleshy tissue known as elaiosomes which ant dispersers consume (Gorb and Gorb, 2003, Gorb and Gorb, 1999). Because plants in NNFR produce fleshy diaspores that are dependent on vertebrates for dispersal, ants in NNFR (like in the Neotropics) can exploit such diaspores to extract food rewards from the soft tissue surfaces. Diaspores with hard surfaces may be more difficult for the ants to access especially if they are not seed predators or granivores.

During this study, ants moved diaspores to their nests and under leaf litter and it was difficult to determine how they exploited the removed diaspores. However, from opportunistic observations of ant-diaspore interactions (detailed description in chapter 3), ants were observed removing the soft fleshy tissues from diaspores (*P. pinnata, C. africanus* and *S. macrocarpa*). These were all diaspores that were too large for them to move. It is, therefore, apparent that they at least exploited those diaspores by consuming the soft and fibrous tissue on them. This is referred to as seed cleaning. Such seed cleaning behaviour by ants has been shown to influence seed germination in the Neotropics (Christianini et al., 2012, Oliveira et al., 1995, Pizo and Oliveira, 2000). It is uncertain whether ants (*Pheidole* sp.1 and *M. opaciventris*) observed interacting with diaspores in NNFR consumed the whole of the small diaspores which they moved, or if they, like myrmecochorous ants, consume the fleshy appendages while leaving the seeds unharmed. However, the fact that 14.5 % of diaspores of *T. orientalis* deposited into ant nests germinated (details is chapter 3) suggests that some of the small diaspores moved to ant nest might be exploited without being harmed.

4.9.4 Diaspore size strongly influences removal by ants

That diaspore size is an important morphological trait limiting seed removal by ants (Christianini et al., 2007) was confirmed in the present study; Ants never moved diaspores that weighed ≥ 0.24 g. They did however, consume the aril and other fleshy material on-the-spot, from diaspores that were apparently too large for them to move (Table 3.1). The most probable explanation for this behaviour is the relatively small size (body size < 0.2 mm) of both ant species observed interacting with diaspores, *M. opaciventris* and *Pheidole* sp. 1, which would make them physically incapable of moving larger diaspores. The implication of this size limitation for regeneration in NNFR is that only small-seeded plant species have the potential to benefit from diaspore removal involving *Pheidole* sp.1 and *M. opaciventris*.

An important observation made during this study was that *Pheidole* sp. 1 and *M. opaciventris* did not move some of the diaspores offered them in the removal experiment, even though the weights of these diaspores fell within the range of the other diaspores moved by both ants. This suggests that factors other than size, are influencing ants' choice of seeds. Chemical traits, especially lipid content, have been shown to be important cues mediating ant removal of non-myrmecochorous diaspores (Christianini et al., 2007) and may be influencing ant-diaspore interactions in NNFR.

4.9.5 Diaspore chemical characteristics

From this study, ants showed (from interactions with diaspores) a stronger association to high ash, calcium and carbohydrate content, but a weak association to moisture, crude fat and crude protein content (Figure 4.10) This is surprising because lipid content which is an attribute of fat content is one of the most important chemical traits that attracts ants. This finding contrasts with other studies which found that ant-diaspore interactions were positively associated with lipid/fat content (Christianini et al., 2007, Pizo and Oliveira, 2001). However, in ecosystems where diaspores have a variable range of fat/lipid content, an association between lipid content and ant-interaction might not be obvious (Pizo and Oliveira, 2000). Hence

ant-diaspore interactions within NNFR are likely to be merely opportunistic and perhaps weakly associated with any chemical trait. Pizo and Olivera (2000) found that ants interacted with diaspores that had a broad range of lipid content in an Atlantic forest in Brazil. Most of such interactions in the tropics involve diaspores that lack specific adaptations to ant dispersal (Pizo and Oliveira, 1998). It is possible that ant interactions with diaspores within NNFR are merely opportunistic and not based on their chemical characteristics. However, the lack of an apparent trend in ant-diaspore interactions based on chemical traits in NNFR might be due the fact that just a few plant species and diaspore types involved were available for chemical analysis at the time of this study.

4.9.6 Vertebrate and ant diaspore removal

The impact that ants and vertebrates have on the removal of diaspores from the ground in NNFR did not differ significantly, although removal of diaspores from the open vertebrate depots was slightly higher (mean = 5.02 ± 0.23 s. e) than from the enclosed, ant-only accessible depots (mean = 4.87 ± 0.22 s. e). (Table 4.8). I assumed that diaspore removal from the open vertebrate accessible depot was predation (Christianini et al., 2007, Gallegos et al., 2014). Vertebrates, such as rodents, were observed (from faecal dropping at some of the open depots) as part of the vertebrate removers of diaspores from the open depots and rodents are the most common and important diaspore predators in tropical ecosystems (Fedriani et al., 2004) and some temperate areas such as the United States (Heithaus, 1981, McAuliffe, 1990) and Europe (Jensen, 1985, Sanguinetti and Kitzberger, 2010).

Predation of diaspores by rodents can influence plant community dynamics (Hulme, 1998, Janzen, 1971). The amount of diaspores available for ant removal might be limited by predation by vertebrates (Fedriani et al., 2004, Heithaus, 1981). The fact that ants removed about 55% of available diaspores from the vertebrate excluded depots within 24 hours, means that they can play a significant role in limiting the number of diaspores lost to vertebrate predators in NNFR. Heithaus (1981) showed that more diaspores where lost to rodent predation in the absence of ants. It is apparent from this study that both ants and vertebrates play significant roles in the removal of available diaspores in NNFR.

Ants in the Neotropics have been found to be important in the secondary dispersal of diaspores primarily dispersed by vertebrates (Christianini and Oliveira, 2009, Christianini and Oliveira, 2010, Hulme, 1998). For example, some species of *Pheidole in* some tropical environments play a dual role by dispersing as well as predating on diaspores (Levey and Byrne, 1993). Their role as diaspore removers of diaspores from beneath parent trees and within the landscape is of potential benefit to such plants. Removal of diaspores by predators presents an opportunity for survival by avoiding density-dependent mortality in instances when they are intentionally or accidentally dropped when transported (Beattie, 1985, Crawley, 2000) or when predators become satiated which might lead to them leaving diaspores unharmed. In the current study, it was not established whether the ants (*Pheidole* sp.1 and *M. opaciventris*) observed removing diaspores within NNFR, are predators or dispersers.

The removal of diaspores from the open vertebrate accessible depots and enclosed ant accessible depots varied by season. Both ants and vertebrates removed more diaspores during the wet season than the dry season. This was not surprising as there is usually a substantial seasonal variation in diaspore availability in tropical ecosystems with more diaspores being available in the wet season (van Schaik et al., 1993). As such, most animals that forage on diaspores are likely to forage more at times of the year when they are available. Small invertebrates such as ants, on the other hand, are likely to forage when the weather is moist as part of their desiccation avoidance strategies (Azcárate et al., 2007, Kaspari and Weiser, 2000). This coincides with higher diaspores availability and diaspore removal observed by ants.

Diaspore removal by ants and vertebrates can vary on a spatial scale (Christianini and Oliveira, 2010). In this study, more diaspores were removed from the vertebrate accessible open depots than the closed depots in the edge habitat. This suggests that vertebrates are probably more likely to use the edge habitat more than the open grassland and the more closed forest habitat. This is consistent with the fact that small vertebrates such as rodents, minimise predation risk by foraging in more closed habitats than in open habitats (Bowers, 1990, Bowers and Dooley, 1993, Falkenberg and Clarke, 1998). In the grassland and forest habitat, more diaspores were removed from the ant enclosed depots than the open depots. Ants in the grassland habitat

unlike vertebrates, are not as vulnerable to predation and can efficiently forage in the grassland habitat. The variations observed in the availability of diaspores within the habitat types could also influence the foraging activity of both vertebrates and ants.

4.9.7 Conclusion

Ant-diaspore interactions largely depend on the availability of diaspores. In this study, I found that the frequency of ant-diaspore interactions was higher in the forest habitat particularly in the wet season. This was probably because diaspores were more available in the forest habitat during the wet season. This is because diaspores were more available in the forest habitat during the wet season. Based on this, I speculate that *Pheidole* sp.1 would play a more significant role in diaspore removal relative to *M. opaciventris* due to its ubiquity within NNFR (details in chapter 2) and the lack of seasonal variation in its foraging activity. However, only plants that produce small diaspores within NNFR can be moved by both these ants due to their small body size (<0.2mm). The fact that ants within NNFR interacted more frequently with diaspores that had soft tissues, suggests that ant interactions with diaspores are opportunistic and not specialised but influenced by the presence of easily accessible food reward on available diaspores. Hence, large diaspore (>1g) with soft fleshy tissue can be exploited even if not moved by ants. The apparent lack of variation in the quantity of diaspores removed by ants and vertebrate seed predators suggests that ants can play a significant role in the removal of small diaspores within NNFR. The results also show that ants can remove available diaspore quickly, thereby reducing the proportion of diaspores lost to vertebrate seed predators, especially if the ants are themselves acting as seed predators.

Appendix

Table 0.9: Results of the generalised linear mixed model with binomial distribution showing the effect of habitat, site, season, and their interactions on diaspore availability in NNFR. Significance is denoted by stars at p = 0.05

Fixed effects:							
	Estimate	Std. Error	z value	Pr(>|z|)			
(Intercept)	-2.2907	0.1991	-11.507	< 2e-16	***		
habitatforest	-0.6104	0.3247	-1.880	0.060098	.		
habitatgrassland	-1.4898	0.3362	-4.431	9.37e-06	***		
siteB	0.3210	0.2450	1.310	0.190103			
siteC	-0.1117	0.2668	-0.419	0.675471			
seasonwet	0.4907	0.2340	2.097	0.035994	*		
habitatforest:seasonwet	0.7924	0.3633	2.181	0.029153	*		
habitatgrassland:seasonwet	-0.6593	0.4788	-1.377	0.168524			
siteB:seasonwet	-0.3581	0.2992	-1.197	0.231363			
siteC:seasonwet	-1.2639	0.3623	-3.488	0.000486	***		

Table 0.10: Result of the generalised linear mixed model with Poisson distribution showing a specific effect of habitat, site, season, month and their interactions on the number of diaspores available in NNFR. Significance is denoted by stars at p = 0.05

Fixed effects:

	Estimate	Std. Error	z value	Pr(>|z|)	
(Intercept)	-0.07126	1.07514	-0.066	0.947153	
habitatforest	-0.56787	0.23351	-2.432	0.015021	*
habitatgrassland	0.30875	0.21926	1.408	0.159086	
siteB	-0.14816	0.09078	-1.632	0.102651	
siteC	-0.05872	0.12701	-0.462	0.643846	
seasonwet	1.43432	1.03473	1.386	0.165693	
seed_sppAnthonotha	-1.63539	1.05388	-1.552	0.120714	
seed_sppbean-shaped unknown	-1.56529	0.65482	-2.390	0.016830	*
seed_sppBielschmedia sp.	1.04649	1.10980	0.943	0.345706	
seed_sppBridelia sp.	1.54651	1.07976	1.432	0.152066	
seed_sppCarapa grandiflora	-1.63070	0.79560	-2.050	0.040399	*
seed_sppCeltis gomphophylla	-1.44975	1.06970	-1.355	0.175326	
seed_sppChionanthus	0.34797	0.62600	0.556	0.578305	
seed_sppClausina	0.14127	0.58134	0.243	0.807994	
seed_sppDeslipct	-1.58171	1.05999	-1.492	0.135649	
seed_sppDiospyrus	-1.45581	1.06256	-1.370	0.170659	
seed_sppEntandrophragma	0.38860	1.49273	0.260	0.794611	
seed_sppFicus sp.	2.22973	1.10539	2.017	0.043680	*
seed_sppGarcinia	0.21858	1.48503	0.147	0.882984	
seed_sppGrass seeds	0.97735	1.23289	0.793	0.427934	
seed_sppHMC	-0.55403	0.81206	-0.682	0.495078	
seed_sppIsolona	-1.72211	1.06865	-1.611	0.107075	
seed_sppLandolphia	0.22126	0.44553	0.497	0.619448	
seed_sppLeaguineaceae sp.	1.73223	1.07570	1.610	0.107326	
seed_sppLobelia sp.	0.02007	0.62468	0.032	0.974366	
seed_sppMacaranga monandra	0.35068	0.34129	1.028	0.304167	
seed_sppMistletoe	1.32567	1.23223	1.076	0.282001	
seed_sppNewtonia sp.	1.98340	1.11346	1.781	0.074865	.
seed_sppOxyanthus sp.	0.48458	1.49830	0.323	0.746375	
seed_sppParkia sp.	-1.26495	1.07693	-1.175	0.240156	
seed_sppPaullinia pinnata	-0.32000	0.30289	-1.056	0.290755	
seed_sppPolyscias fulva	0.83047	0.50286	1.651	0.098639	.
seed_sppPsorospermun aurantiacum	0.46734	0.32323	1.446	0.148222	
seed_sppPsychotria sp.	1.18665	1.08175	1.097	0.272654	
seed_sppPuteria	-1.13300	1.06962	-1.059	0.289484	
seed_sppRouvolfia vomitera	-0.29180	0.46250	-0.631	0.528102	
seed_sppRubiacea	0.75899	1.15856	0.655	0.512395	
seed_sppunknown	0.02741	0.34776	0.079	0.937178	
seed_sppVernonia sp.	0.65359	1.12017	0.583	0.559572	
seasonwet:seed_sppBielschmedia sp.	-1.77741	1.22242	-1.454	0.145943	
seasonwet:seed_sppBridelia sp.	-1.75589	1.06503	-1.649	0.099213	.
seasonwet:seed_sppFicus sp.	-3.24271	1.09409	-2.964	0.003038	**
seasonwet:seed_sppLeaguineaceae sp.	-1.97760	1.05547	-1.874	0.060977	.
seasonwet:seed_sppMistletoe	-2.90093	1.57489	-1.842	0.065477	.
seasonwet:seed_sppNewtonia sp.	-3.09096	1.12910	-2.738	0.006190	**
seasonwet:seed_sppPsychotria sp.	-0.93912	1.05172	-0.893	0.371890	
seasonwet:seed_sppRubiacea	-0.62787	1.13627	-0.553	0.580558	
habitatforest:seasonwet	0.84562	0.25266	3.347	0.000817	***
habitatgrassland:seasonwet	-0.79175	0.30393	-2.605	0.009187	**

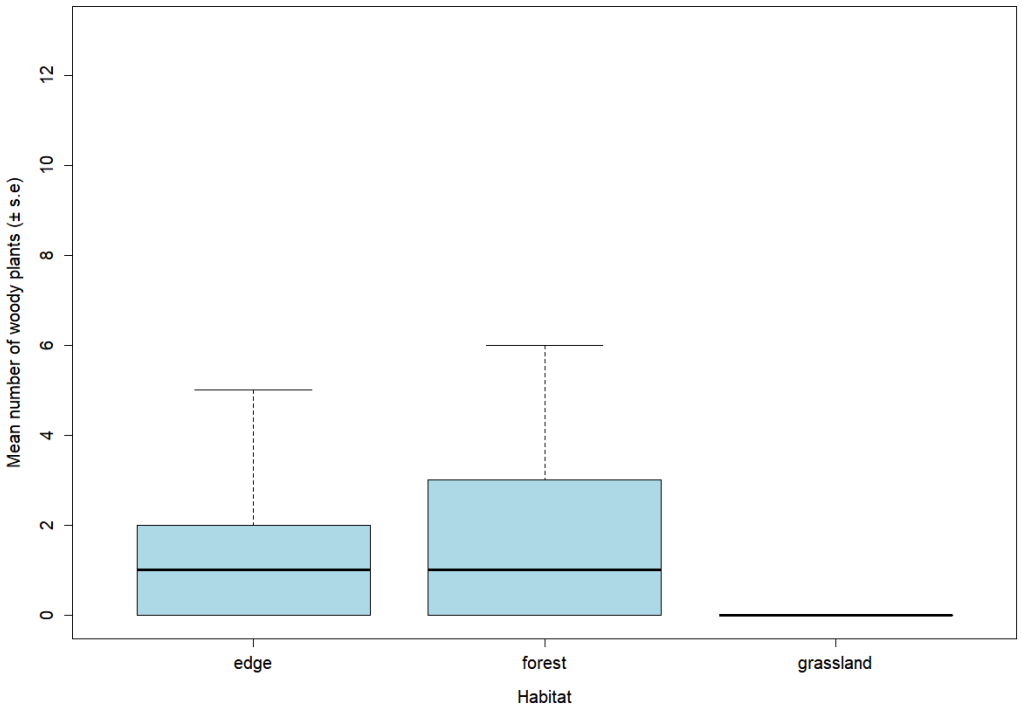

Figure 0.8: Mean number of woody plants species in $2m^2$ plots among the grassland, edge and forest habitat. Grassland: n =558, edge: n = 1488, and forest: n = 372.

Chapter 5: Do ants affect seed germination success and soil properties in Ngel Nyaki Forest Reserve?

Abstract

Ant-diaspore interactions have been studied in temperate, sub-tropical and tropical areas of the world. While studies in temperate, and sub-tropical areas of the world focus more on true myrmecochory which is a mutualistic ant-diaspore interaction involving diaspores that are adapted to dispersal by ants, recent studies in tropical parts of the world reveal beneficial interactions between ants and diaspores not adapted to dispersal by ants. Ground-dwelling ants are also known to affect soil properties and their impact on soils as well as interactions with diaspores, vary in different ecosystems. However, information on what effect ants have on seeds not adapted to dispersal by ants, as well as soils in West Africa, is lacking. In Ngel Nyaki Forest Reserve, two ground dwelling ant species (*Pheidole* sp 1. and *Myrmicaria opaciventris*) from the sub-family Mrymicinae have been found to interact with diaspores of 10 different plant species. However, their impact on surrounding soil and the effect of their interactions on the fitness of plants is yet to be explored and understood. Physical and chemical characteristics of soil from ant nests and non-nest soils were compared. Controlled experiments were used to investigate ant seed cleaning rate using *Paullinia pinnata*. Radicle emergence rate and growth of cleaned and intact diaspores of *Paullina pinnata* were compared in germination experiments. The effect of ant nest soil on seedling growth was also compared using seeds of *Vigna unguiculata* planted in ant nest soil and non-nest soil from the forest edge as a control. There was a significant difference in chemical and physical properties between soil from ant nests and non-nest soil. (Pillai = 0.45; df = 1, $p < 0.0001$). Cleaned seeds of *Paullinia pinnata* germinated faster than intact seeds ($G = 4.55$, df = 1, $p < 0.05$) and seedlings from cleaned seeds had longer shoot length and higher wet weight than seedlings from intact seeds. Ant nest soil had a positive effect on the shoot length and wet mass of seedlings of *Vigna unguiculata* but did not affect root length. Results from this study show that seed cleaning by ants significantly affects seed germination and seedling growth of *Paullinia pinnata*. This shows that ants can

aid seed germination and seedling growth in NNFR. Additionally, ants altered soil physical properties, which had a positive effect on seedling growth. Suitable microsites, early germination and predation avoidance are crucial for natural regeneration, especially in degraded tropical ecosystems.

5.1 Introduction

Consumption of arils and fleshy fruit material from diaspores that fall spontaneously from parent trees or that have been dispersed by frugivorous vertebrates, is an interaction that has been observed between ants and non-myrmecochorous diaspores in the Neotropics. It may occur on the spot where diaspores are encountered (Leal and Oliveira, 1998, Pizo and Oliveira, 1998), or following transport of diaspores into ant nests and is commonly referred to as seed cleaning (Oliveira et al., 1995, Pizo and Oliveira, 1998).

This activity by ants has been shown to reduce or prevent pathogen attack on diaspores, possibly because ant salivary secretions inhibit the development of fungal and bacterial spores on diaspores (Beattie et al., 1986, Fernández-Marín et al., 2006). Fungi and pathogens cause significant seed mortality in tropical ecosystems due to the warm, humid conditions in these ecosystems, which enhance pathogen growth (Augspurger, 1990). Myster (1997) found that more seeds were lost to pathogenic infection than to granivory in a north-east Puerto Rican forest. Such mortality constitutes an important barrier to seed germination and seedling recruitment. Hence, diaspore cleaning by ground-dwelling ants can enhance seed germination and plant recruitment by preventing seed mortality caused by pathogens (Gallegos et al., 2014, Giladi, 2006, Lambert et al., 2005).

Ground dwelling ants also exert considerable impact on the surrounding soil environment through their foraging activities and nest building (Cammeraat and Risch, 2008, Dean and Yeaton, 1993, Nkem et al., 2000). They have been described as ecosystem engineers (Dostál et al., 2005, Folgarait, 1998, Frouz and Jilková, 2008), exhibiting a capacity to alter physical, chemical and biological properties of soils (Dostál et al., 2005, Nkem et al., 2000) through the accumulation of organic

material and bioturbation (Frouz and Jilková, 2008). Physical alteration of the soil by ants involves burrowing to build nest structures where food is deposited, and organic matter is accumulated (Cammeraat and Risch, 2008, Frouz and Jilková, 2008). Ant burrowing breaks up the soil and increases porosity, which enhances penetrability (Cammeraat and Risch, 2008, Passos and Oliveira, 2004). Some ant species also build above-ground structures by removing soil and organic material from the surroundings which gives rise to below-ground vertical tunnels and horizontal chambers for food storage (Frouz et al., 2003, Frouz and Jilková, 2008).These structures are believed to control microclimate conditions such as temperature and moisture within the nest chambers (Frouz and Jilková, 2008). Alteration of the chemical properties of soil by ants may increase macro-nutrient (phosphorus and nitrogen) content in soil and shift the soil pH towards neutral levels (Cammeraat and Risch, 2008, Frouz and Jilková, 2008). For example, soils from nests of leafcutter ants were observed to contain a significantly higher concentration of macronutrients than non-nest soil locations (Farji-Brener, 2000, Farji-Brener and Werenkraut, 2015, Moutinho et al., 2003).

Changes in soil properties due to ant activity, may provide suitable growth conditions for vascular plants (Dean et al., 1997) and diaspores are likely to benefit from improved seed germination and seedling growth in such conditions (Beattie and Culver, 1983, Farji-Brener and Werenkraut, 2015, Frouz et al., 2003). The ant nest environment has been shown to enhance seed germination and seedling survival (Beattie and Culver, 1983, Culver and Beattie, 1980, Dean and Yeaton, 1992). Thus, if seed cleaning occurs within ants' nests, seed survival, diaspore germination and subsequent growth and survival of seedlings could be positively affected through the avoidance of predation, prevention of pathogen attack, and improved soil conditions.

Despite the potential of seed cleaning and alteration of soil conditions by ant species to promote natural regeneration, it is possible, and has indeed been shown, that ant species do not all alter soil conditions (Verchot et al., 2003) in the same way or have similar effects on germination success through seed cleaning (Lafleur et al., 2005). However, like other interactions between ants and non-myrmecochorous seeds, these questions have not been investigated together in Afrotropical forest ecosystems

where there is an abundance of ant species and many plants with fleshy diaspores. So, in this chapter, I investigated the effect of seed cleaning by *Myrmicaria opaciventris* and *Pheidole* sp.1 on germination success using *Paullinia pinnata*, which they both interact with by consuming the fleshy aril covering the seed. The alteration of soil properties by both species and the effect on seed germination was also investigated

5.2 Objectives

The objectives of this study are to ascertain if *Pheidole* sp. 1 and *M. opaciventris*:

i. improve germination success of *P. pinnata* through seed cleaning
ii. affect soil physical and chemical properties which in turn increase growth of plants (using *Vigna unguiculata* seeds)

The null hypotheses were as follows:

- Diaspore cleaning by *Pheidole* sp.1 and *M. opaciventris* does not have any effect on radicle emergence of diaspores or seedling growth of *P. pinnata*
- There is no difference in the physical and chemical properties of soil from the nests of *Pheidole* sp.1, *M. opaciventris*, and surrounding non-nest soil.
- Soil from nests of *Pheidole* sp.1 and *M. opaciventris* and surrounding non-nest soil have similar effects on plant growth.

5.3 Methods

5.3.1 Study area

The site of the study Ngel Nyaki Forest Reserve (NNFR), has already been described in detail in Chapter 1. Seed cleaning experiments were conducted in the edge habitat of the forest.

5.3.2 Study species

Diaspores of *Paullinia pinnata L.*(Sapindaceae) were used for the seed cleaning experiment. This species was chosen because of the availability and abundance of

diaspores at the time of the study, and also because ants had been observed cleaning these diaspores during the reconnaissance survey of opportunistic ant-diaspore interactions in Chapter 3.

Paullinia pinnata is a woody vine found in Africa and South America (Zamble et al., 2006) and in places where it is present, it often occurs at forest edges (Chapman et al., 2001). Its leaves have prominent veins with five serrated finger-like leaflets (Zamble et al., 2006) and fruits are dark pink or red when ripe, bearing two to three white arillate seeds that have a single black stripe. When the fruit of *P. pinnata* ripen, they open and are exposed on the plant. Some of the fruit is eaten off the seeds by frugivores on the tree, and some seeds are released from the parent plant by ballistic expulsion from the pod. Primates also disperse diaspores of *P. pinnata* (Mascaro et al., 2004) and monkeys within NNFR have been observed removing and eating the diaspores from the plant (Bawuro, *pers.com*). The average wet weight of the arillate diaspores in NNFR was determined to be 0.34 g (chapter 4).

Figure 0.1: Ripe fruits of *Paullinia pinnata* L. (Sapindaceae) (Photo by Tom Hollowell adapted from google images).

5.3.3 Assessment of the cleaning rate of *Paullinia pinnata* by *Pheidole* sp. 1 and *Myrmicaria opaciventris*

A five-day census was used to monitor the rate at which ants cleaned seeds of *P. pinnata* at the forest edge. Seed depots comprised a single seed placed on white filter paper. Each depot was protected with a mesh cage (0.5 x 0.5 cm mesh size) placed over the diaspores and dug into the ground to exclude vertebrates that might prey on, or remove the diaspores (Christianini and Oliveira, 2010, Hughes and Westoby, 1992, Oberrath and Böhning-Gaese, 2002). Depots were placed within the edge habitat from the outermost canopy trees (0 m), and at 10 m intervals up to 40 m into the forest, to explore the effect of distance from the forest edge on ant attendance. Depots placed at 0, 10, and 20 m from the outermost canopy tree were categorised as "close to the edge", while depots placed at 30 and 40 m from the outermost canopy tree were categorised as "far from the edge". Depots were laid out at 0600 hrs and checked for seed cleaning on days 1, 2, 3 and 5. The species and number of ants attending seeds were recorded during each period of observation. The percentage of aril removed from the seeds was estimated visually and used to categorise diaspores into six classes: 0, 1-25, 25-50, 51-75, 76-99 and 100% cleaned, after the method of Pizo and Oliveira (1998).

5.3.4 Nursery germination experiment: the effect of cleaning on germination of *Paullinia pinnata* diaspores

A nursery germination experiment using diaspores of *P. pinnata* was used to compare the emergence rate of the radicle, its growth rate, and total biomass of germinated seedlings from intact and cleaned diaspores manipulated by manually removing all arils (mimicking ant handling). Germination experiments were carried out between June and August 2016 during the wet season. and included two different diaspore treatments of *P. pinnata*: Treatment 1- Intact seed: released through ballistic expulsion from the parent plant and unattended by ants. Treatment 2- Thoroughly cleaned seed: prepared by manually stripping the seed of all its aril (fleshy tissue) mimicking cleaning by *Phiedole.* sp 1 and *M. opaciventris*.

Eighteen seeds from each treatment were planted two inches deep into separate 6-litre black poly pots filled with compost. Pots were placed in the outdoor plant

nursery and because it was the wet season and there was consistent rain, no watering was required. Planted seeds were checked for germination every ten days, for a total period of 40 days. Sixty days after planting, the number of leaves on germinated seedlings in each treatment were counted, and the length of each shoot was measured with a ruler. After this, vegetative parts (shoots and roots) were harvested. Root lengths were measured, and the fresh weights of shoots and roots were measured separately and recorded.

5.3.5 Physical and chemical properties of soil from ant nests and surrounding soil.

To determine the effect of ants on soil properties, 100 g of soil was collected using a garden spade from a depth of 5 cm in the middle of the nests of each of *Pheidole* sp.1 (N = 20) and *Myrmicaria opaciventris* (N =18). The same quantity of soil was taken from a 5 cm depth of non-nest soil at least 2 m distance from the edge of each ant nest as a control. Soil samples for both ant species and alternate non-nest controls were taken from the forest, edge and grassland habitats. Soil temperature and moisture of ant nest and off-nest soil were measured using a general tool T300-36® soil thermometer (measures 1°C difference) and Gardman® (model GM16070) pH and moisture meter (0-10 moisture gradient range). Soil penetrability was measured using a machete stuck into the nest and alternate non-nest soil till it reached the point of resistance, and the depth of penetration was measured using a metre rule. Soil samples were then air dried for 48 hours, packaged in brown paper bags and sent to the Agronomy laboratory, University of Ibadan, Nigeria for determination of pH, Nitrogen (N), Phosphorus (P), Potassium (K), and organic Carbon content.

5.3.6 Effect of soil from ant nests on plant growth

The effect of ant nest soil and non-nest soil on plant growth were compared in a germination experiment using the common cowpea *Vigna unguiculata* Leguminosae also called black-eyed beans. This species was used because it has a fast growth rate, which allowed for quick and easy assessment of vegetative structures. A pair of black-eyed beans was planted in individual holes of the six-hole grow trays used for the experiment within the NNFR nursery. A pair of seeds was planted in 30 grow seed trays holes containing ant nest soil, and 30 holes containing non-nest soil from

forest edge (as control). The trays were watered every two days for fifteen days, after which the shoot and root lengths of germinated seedlings were measured (cm).

5.4 Statistical analysis

Analysis were carried out using R studio version 3.2.0 (R Core Team 2015). All analysis with $p < 0.05$ were considered significant.

5.4.1 Seed cleaning rate of *Paullinia pinnata*

Seed cleaning rate of *P. pinnata* was illustrated using pie charts. The charts show how effective ants are in cleaning the seed on the forest floor over time (day 1, 2, 3 and 5). The census was summarised based on three seed categories;

Intact seeds – seeds which were unattended to with 0% of its aril missing after at least 24-hours

Still being cleaned – seeds that had between 1% - 75% of aril missing

Cleaned - seeds that had between 76-100% of aril missing

Chi-square test was used to examine whether ant attendance to diaspores and cleaning rate differed between diaspores placed “close to the edge” and those placed “far from the edge” while an independent t-test was used to test the significance of the difference in the numbers of *Pheidole.* sp 1 and *Myrmicaria opaciventris* cleaning diaspores of *P. pinnata*.

5.4.2 Effect of seed cleaning on *Paullinia pinnata* germination and seedling growth

The G-test of independence was used to determine if there was a significant difference in the proportion of cleaned and intact diaspores that germinated. Shoot length, root length, number of leaves and weight of seedlings from intact and cleaned seeds were compared using Wilcoxon test.

5.4.3 Effect of soil from ant nest on plant growth

An independent sample t-test was used to compare shoot length and root length of germinated seedlings from soil from ant nest and control soil.

5.5 Results

5.5.1 Seed cleaning rate of *Paullinia pinnata*

A total of 80 *P. pinnata* seeds were used to assess how capable ants were, at cleaning this diaspore on the forest floor. The proportion of cleaned seeds versus those left unattended or partly cleaned after days 1, 2, 3 and 5 of the experiment are summarised in Figure 5.2, while Figures 5.3a and 5.3b respectively, show an uncleaned/intact and a cleaned *P. pinnata* seed.

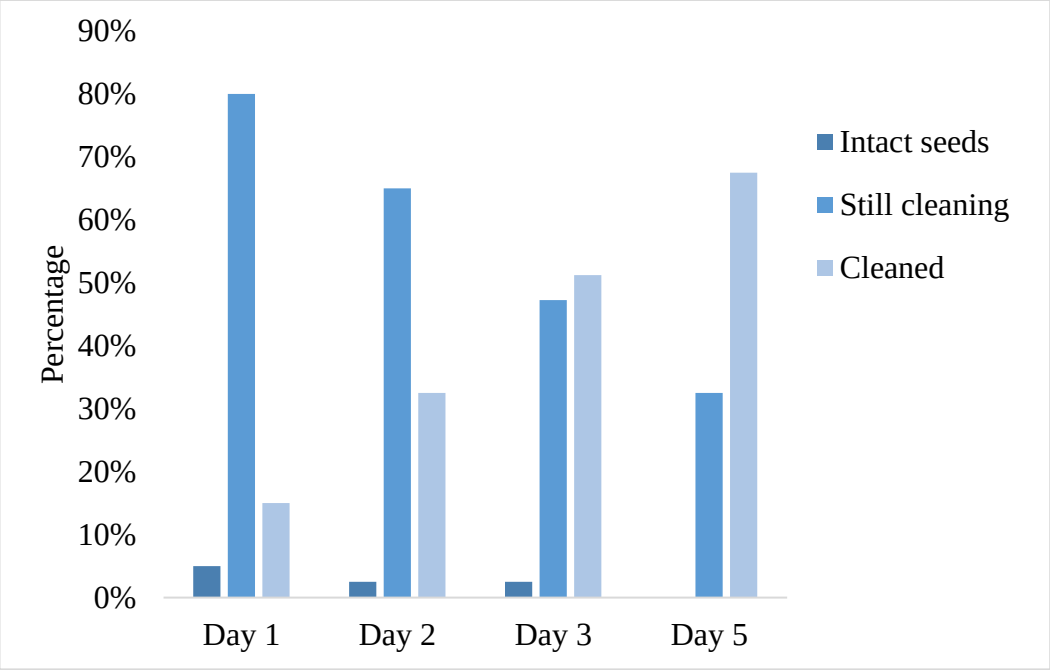

Figure 0.2: Seed cleaning rates of *Paullinia pinnata* by ants, over a five-day period (Number of seeds = 80).

(a)

(b)

Figure 0.3: (a) Intact seed with aril being cleaned; (b) cleaned seed with aril fully removed.

Distance of diaspore depot in relation to the edge (close or far) had no effect on the relative proportions of cleaned, partly cleaned and uncleaned diaspores (χ= 6, df= 4, p =0.19). This shows that ants clean seeds at similar rates irrespective of diaspore location (Figure 5.4).

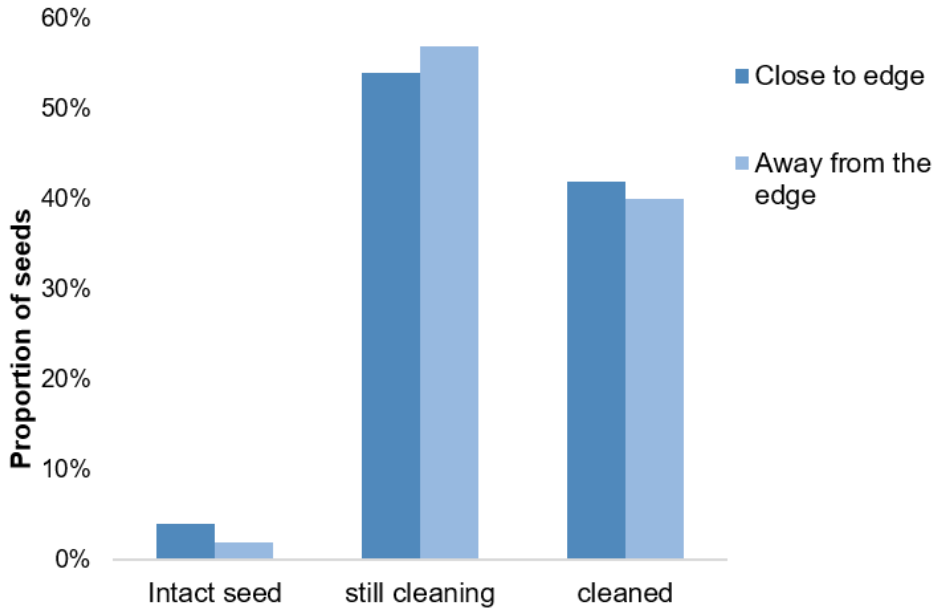

Figure 0.4: Ant-attendance and non-attendance to diaspores at different locations. (Close to the edge: n = 48, Away from the edge: n = 32).

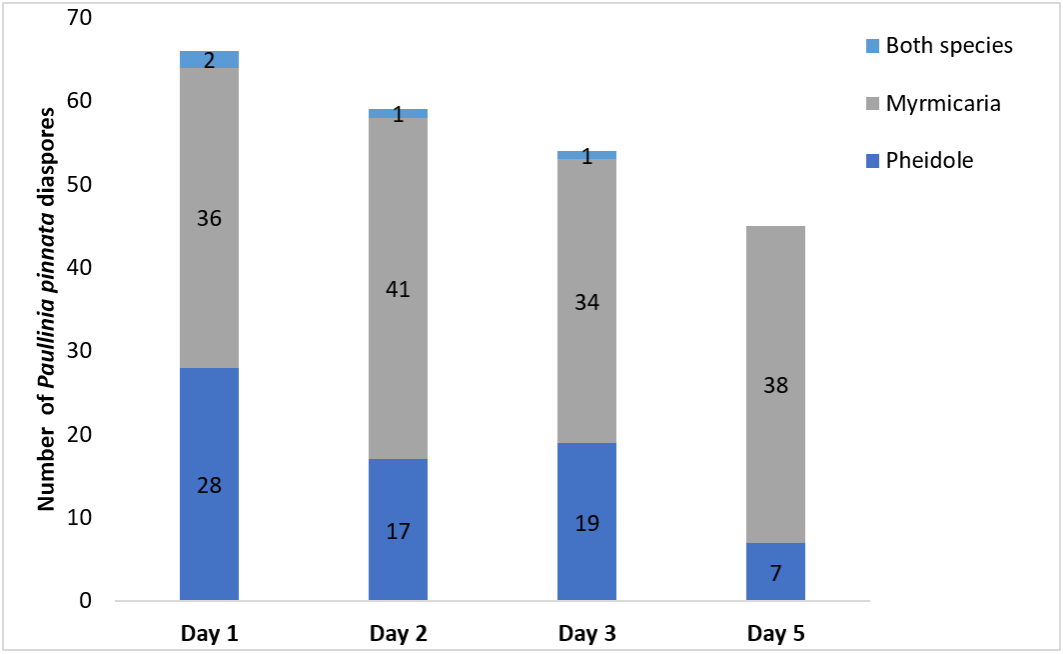

Figure 0.5: Ant species attendance to diaspores of *Paullinia pinnata* over five days. Labels on bars indicate number of depots where *Pheidole* sp. 1 and *Myrmicaria* attended seeds either separately or together.

The number of ants (*Pheidole.* sp 1 and *M. opaciventris*) observed cleaning diaspores of *P. pinnata* during the five-day seed cleaning census differed significantly between the two-ant species (t = 4.27, df = 6, p-value < 0.005) with a mean of 37.35 ± 1.49 for *M. opaciventris* and 17.75 ± 4.31 for *Pheidole.* sp 1. There was little variation in the average number of *M. opaciventris* attending seed depots over the five days while *Pheidole* numbers had declined by day 5 (Table 5.5).

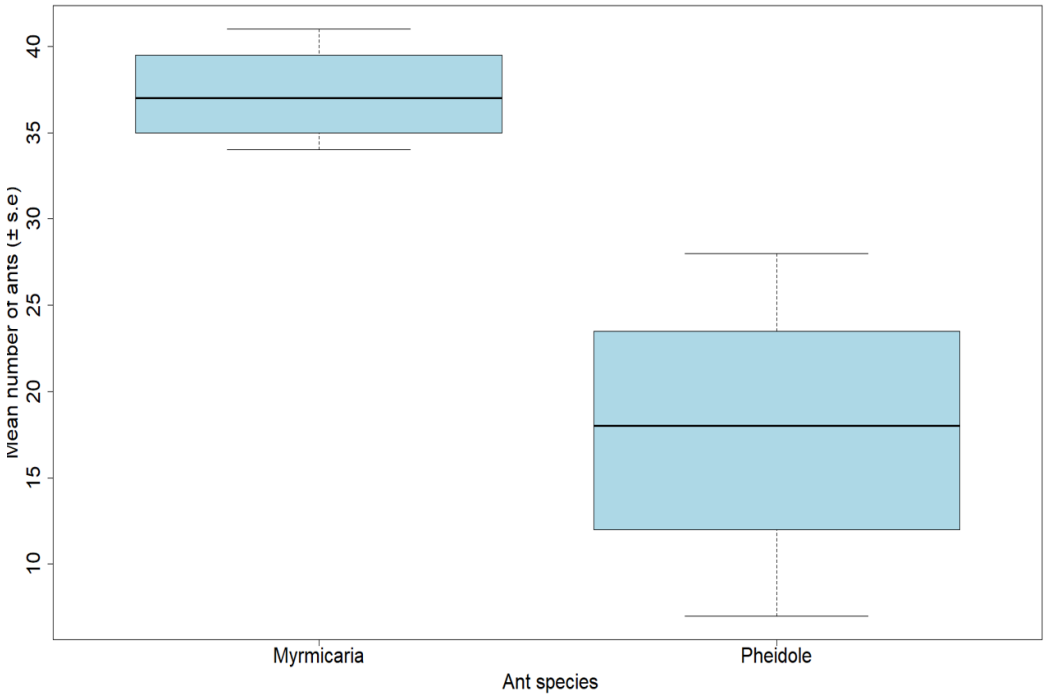

Figure 0.6: The mean number of *Myrmicaria opaciventris* and *Pheidole* sp.1 cleaning *Paullinia pinnata* during 5-day seed cleaning experiment.]

5.5.2 Effect of seed cleaning on germination rate

Cleaned seeds of *P. pinnata* germinated faster than intact diaspores (G = 4.55, χ^2df = 1, p-value <0.05). Sixty five percent (65%) of cleaned seeds germinated after 40 days of planting while 45% of intact seeds germinated in the same period.

5.5.3 Effect of cleaning on growth of *Paullinia pinnata* seedlings

Shoot length of seedlings from cleaned seeds and intact seeds differed significantly (W = 270, p < 0.001). Cleaned seeds had a mean shoot length of 6.17 ± 0.28 (s.e) cm

while intact seeds had a mean shoot length of 4.71 ± 0.26 (s.e) cm. The number of leaves of seedlings from cleaned seeds and intact seeds was also significantly different (W = 217.5, p < 0.05), of which clean seeds had more leaves than intact seeds. Mean wet weight of seedlings from cleaned seeds was significantly greater than that of seedlings from intact seeds (W = 157.5, p = 0.02). While seedlings of cleaned seeds had a mean wet weight of 1.21 ±0.14 g, those from intact seeds had a mean wet weight of 0.78 ± 0.13 g. In contrast, the root lengths of seedlings of intact and cleaned seeds did not differ significantly. (t = 0.89, df = 25.3, p = 0.37). Mean root lengths were 24.3 ± (s.e) 1.22 cm and 22 ± (s.e) 1.66 cm for seedlings from cleaned and intact seeds respectively (Figure 5.7).

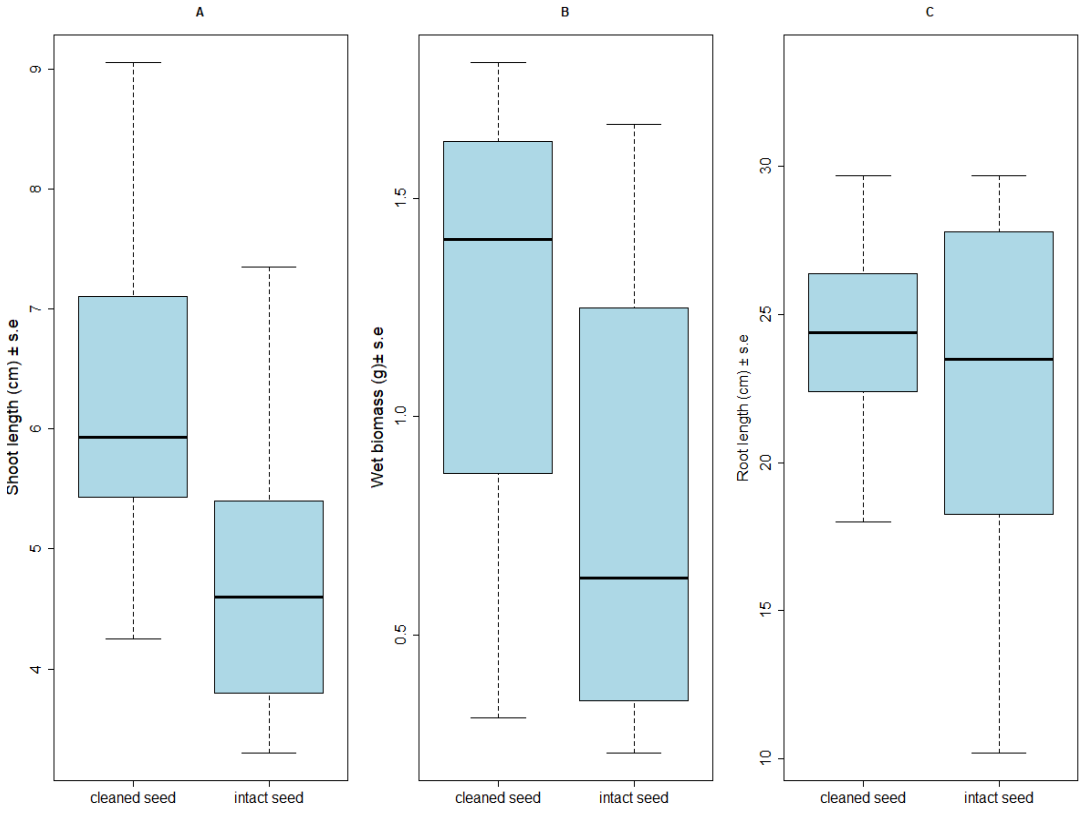

Figure 0.7: A — shoot length (cm), B — wet weight (g) and C — root length (cm) of seedlings from cleaned and intact seeds of *P. pinnata*. (N = 18 for cleaned seeds, N=18 for intact seeds).

5.5.4 Physical and chemical properties of *Pheidole* sp. 1 and *Myrmicaria opaciventris* nest soils and surrounding non-nest soil

M. opaciventris and *Pheidole* sp.1 significantly altered physical and chemical properties of the soil within their nests (Pillai = 0.76, df = 2; $p < 0.0001$). Soil from ant nests was more penetrable than non-nest soil but they had significantly lower phosphorus content than non-nest soils. In contrast, soil pH, soil nitrogen, potassium, organic carbon content, soil temperature and soil moisture content did not differ between soil from *M. opaciventris* nest, *Pheidole* sp.1 nest, and non-nest soil (Table 5.1).

Table 0.1: Results of Multivariate analysis of variance comparing the physical and chemical properties of soil from nests of *Pheidole* sp. 1 and *Myrmicaria opaciventris* with surrounding non-nest soil

Variable	**df**	**F-value**	**P-value**
Penetrability	*2*	15.0	< 0.0001*
Phosphorus	2	7.21	<0.01*
Organic carbon	2	1.29	0.28
Potassium content	2	1.42	0.24
Total Nitrogen	2	0.93	0.39
Temperature	2	0.32	0.72
Soil pH	*2*	2.31	0.10
Moisture content	2	1.12	0.32

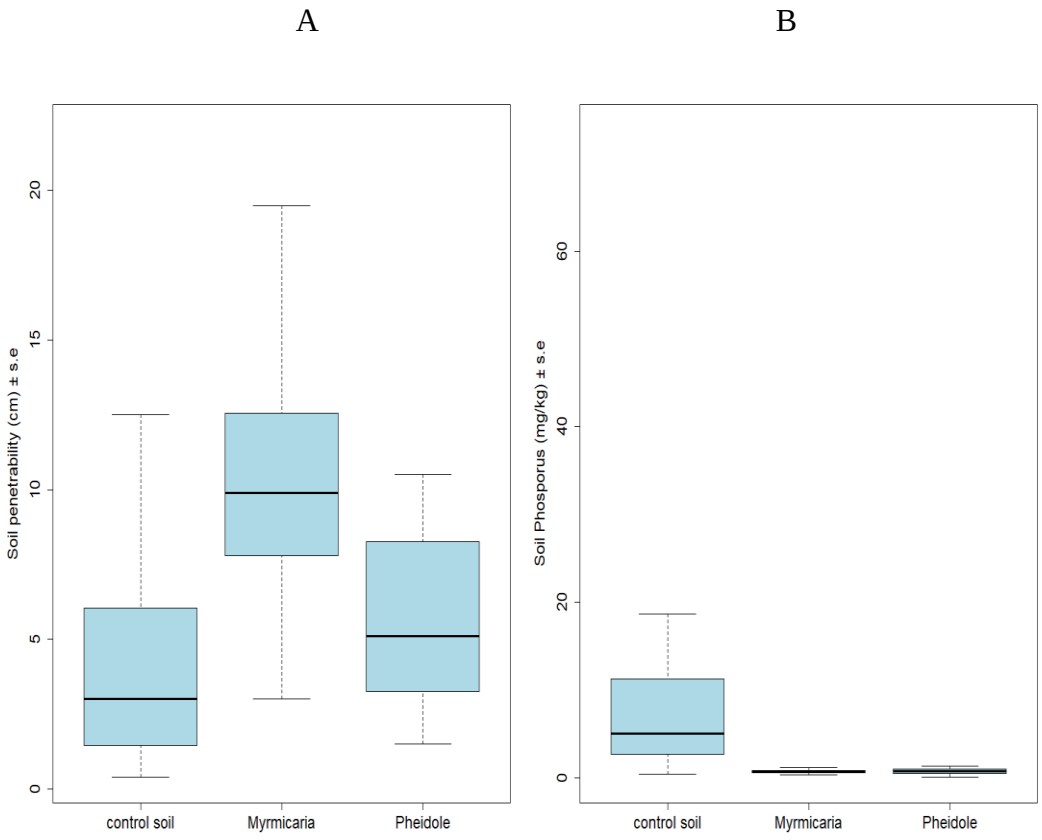

Figure 0.8: A — soil penetrability, B — soil phosphorus. Control soil: n = 38, *Myrmicaria opaciventris*: n = 18 and *Pheidole* sp. 1: n = 20

5.5.5 Effect of soil from ant nest on plant growth

Ant nest soil had a significant positive effect on the growth of *Vigna unguiculata*. Seedlings produced by seeds sown in ant nest soil had significantly longer shoots than their control soil (non-nest) counterparts ($t = 2.58$, $df = 20$, $p < 0.05$); Figure 5.8 (C). In contrast, this response was not found in root length and there was no significant difference in the root length, Figure 5.9 (D) of seedlings from ant nest soil and the control soils ($t = -0.09$, $df = 20$, $p = 0.92$).

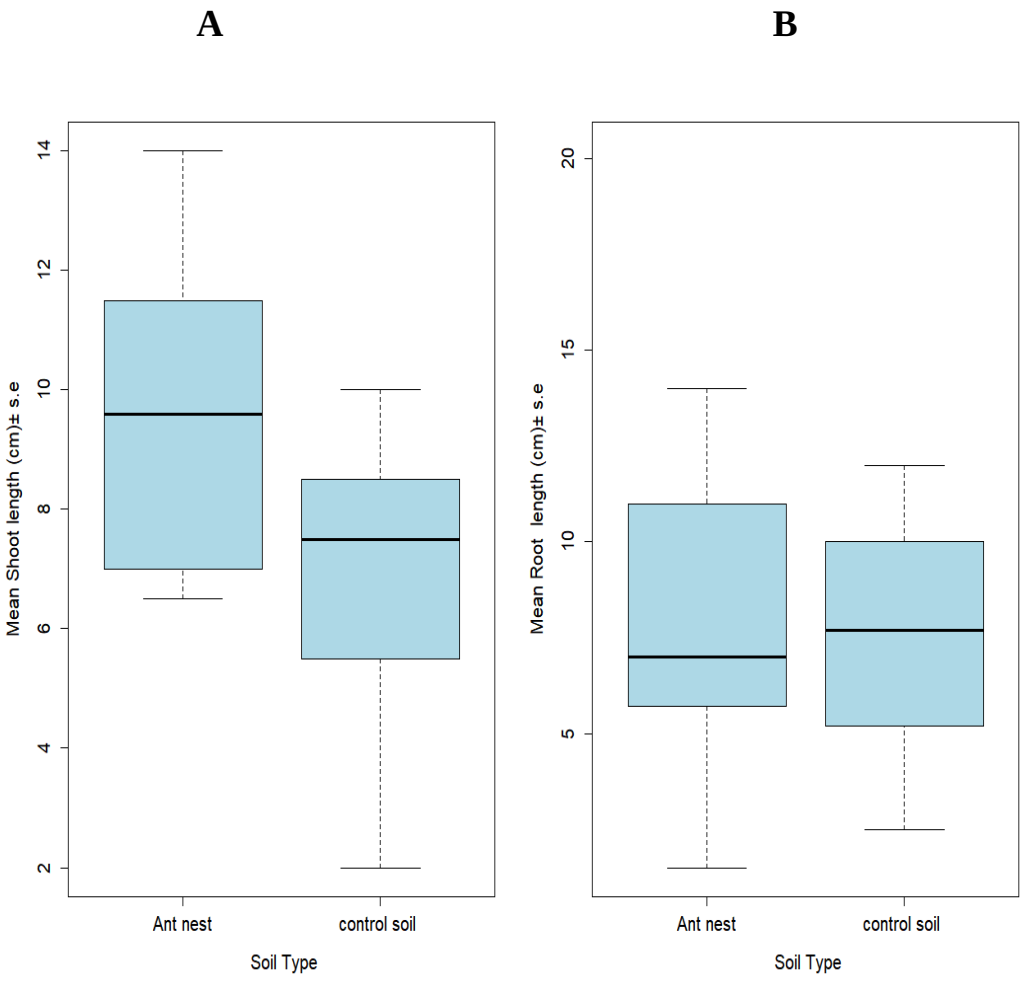

Figure 0.9: A— shoot length (cm), B — root length (cm) of *Vigna unguiculata* seedlings from ant nest and control soil. Ant nest: n = 30, control soil: n= 30.

5.6 Discussion

Seed cleaning by ants has been demonstrated to be beneficial in reducing diaspore mortality due to fungal attack, and previous studies have shown that diaspores with fleshy appendages are more susceptible to fungal attack than cleaned ones (Myster, 1997, Ohkawara and Akino, 2005). At the end of the five-day seed cleaning census, none of the 80 experimental diaspores of *P. pinnata* was left unattended, and a considerable proportion (70%) of the seeds had been cleaned (Figure 5.3b) of all fleshy tissue (aril) by *Pheidole* sp. 1 and *M. opaciventris* (Table 5.1). This demonstrates that a significant proportion of fallen diaspores of *P. pinnata* within

NNFR, are effectively cleaned by these ants and may receive the benefit of reduced fungal attack, although this was not investigated.

Pheidole sp.1 and *M. opaciventris* did not move the diaspores of *P. pinnata* and all diaspores placed at depots were accounted for. Instead, both species appear to recruit workers to forage collectively on the diaspores. The mean number of *M. opaciventris* cleaning diaspores of *P. pinnata* at seed depots, was significantly higher than that of *Pheidole* sp.1. and could be because *M. opaciventris* is more abundant than *Pheidole* in the edge habitat where the experiment was carried out (details in chapter 2). The observation that each species preferred to attend to diaspores at separate depots, and rarely foraged at the same seed depot, implies a spatial partitioning in the use of this resource by the two species. This probably means that both ants can spend more time effectively cleaning seeds than competing, which is to the benefit of the plant.

5.6.1 Seed cleaning by ants improves germination of *Paullinia pinnata*

Seed germination is an important process affecting the ecology of plants because the time at which a plant germinates, can have a significant impact on its fitness (Baskin and Baskin, 1998). Seed cleaning by ants has been shown to shorten germination time and increase the proportion of seeds germinating (Christianini et al., 2007, Oliveira et al., 1995). In this study, cleaning of *P. pinnata* diaspores did not appear to shorten germination time, but cleaned seeds eventually germinated at a faster rate than intact or uncleaned diaspores, demonstrating that cleaning of *P. pinnata* diaspore by ants increased seed germination rates and could probably affect seedling recruitment within NNFR. Previous studies have shown the positive effect ant diaspore-cleaning on germination rates and success (Christianini et al., 2007, Guimarães Jr and Cogni, 2002, Oliveira et al., 1995, Passos and Oliveira, 2003). Faster germination rates of seeds can reduce vulnerability to mortality from succeeding seasons of drought and so, seedlings that germinate faster have a better chance of recruitment, especially in competitive environments where there is seasonal variability in moisture (Orrock and Christopher, 2010). Faster germinating diaspores are also more likely to avoid predation because of the limited period of

exposure to seed predators (Bowers and Dooley, 1993, Christianini and Oliveira, 2010).

5.6.2 Ants alter physical and chemical properties of soil to a limited extent

Highly compact soil can limit seed germination, root penetration, and overall plant establishment and growth (Nkem et al., 2000). Ground dwelling ants may build mounds with perforated and interconnected galleries beneath the soil where they accumulate food and reproduce (Cammeraat and Risch, 2008, Folgarait, 1998, Hölldobler and Wilson, 1990, Jouquet et al., 2006). This can significantly affect soil biota and influence belowground biomass and growth of vascular plants (Dean et al., 1997, Nkem et al., 2000). In the current study, *Pheidole* and *M. opaciventris* significantly improved soil penetrability, which could potentially benefit seeds deposited in the nests of these ants.

The effect of ants on soil chemical properties depends on the properties of surrounding soil as the alteration of chemical properties is dependent on the available supply in the soil (Frouz et al., 2003). In this study, ants did not affect most of the soil chemical properties tested, except phosphorus content, which was significantly lower in ant nests compared to surrounding soil. This is in contrast with most studies that found higher chemical content in ant nests than in surrounding soils (Farji-Brener and Werenkraut, 2015, Frouz et al., 2003). It has been shown that ant activity from the accumulation of food and faecal matter improves soil chemical properties (Dostál et al., 2005, Folgarait, 1998, Frouz et al., 2003). Particularly, the potassium content in soil, has been found to increase in ant nest soil irrespective of its content in surrounding soils (Frouz et al., 2003).

The influence of ants on soil chemical properties is known to also depend on the age of the ant nest (Frouz et al., 2003, Frouz and Jilková, 2008). The limited impact on soil chemical properties by *Pheidole* sp.1 and *M. opaciventris* within NNFR, may be connected to the length of time their nests persist. It is known that some ant nests undergo frequent structural changes over a short period because they excavate new galleries while abandoning old ones (Hughes, 1991) and if *Pheidole* sp.1 and *M.*

opaciventris exhibit such behaviour, their impact on soil chemical properties would likely be limited.

Ants that build large conspicuous mounds usually occupy such nests for years (Beattie and Culver, 1983) and may alter soil chemical properties than short-lived nests. Nests occupied for less than two years usually do not vary in soil chemical properties with surrounding soils (HUGHES, 1991). *M. opaciventris* was observed to build small nests with a conspicuous mound, while *Pheidole* sp.1 built small and inconspicuous nests that were not easily located. The fact that nests of both *Pheidole* sp.1 and *M. opaciventris* are small suggest that they are not occupied for long periods of time and might explain why they did not have a noticeable effect on soil chemical properties in NNFR. They also did not appear to alter soil moisture content. The amount of moisture retained in soil is dependent mainly on the organic matter content (Danalatos et al., 1995, Rawls et al., 2003). From this study, there was no significant difference in soil organic matter content between the ant nest soil and non-nest soil. As such, it is not surprising that moisture content between ant nest soil and surrounding soil was also not different. Although ants appeared not to affect soil chemical properties remarkably, soil from ant nest influenced the growth of *Vigna unguiculata* seeds. Seedlings grown on ant nest soil had longer shoots than seedlings grown on soil from the surrounding forest. The roots of seedlings grown on soil from ant nest and those grown on surrounding soil did not differ significantly. It is possible that the difference in the texture of the soil from ant nests (Appendix- Table 5.2) was responsible for the positive effect of plant growth. This is because plant nutrient and water uptake from more compact soils is limited which can affect seed germination and inhibit seedling growth (Kozlowski, 1999).

5.6.3 Conclusion

Results of this study confirm that diaspores within NNFR benefit from seed cleaning by ants which positively affects germination rate and plant growth significantly. In other studies, it has been shown to reduce seed mortality caused by pathogenic infestation (Ohkawara and Akino, 2005, Oliveira et al., 1995) and it would be important to determine in the future, whether this also occurs in NNFR. Additionally, this study shows that ants in NNFR affected soil physical properties by increasing

penetrability which can enhance germination and seedling growth. Ants in NNFR could therefore be providing suitable microsites for germination leading to earlier germination and these are factors that are necessary for natural regeneration of plants, especially in degraded tropical ecosystems.

5.7 Appendix

Table 0.2: Properties of ant nest and non-nest surrounding soils

Soil Properties	**Ant Nest**		**Non-nest**	
	Mean	**± s. e**	**Mean**	**± s. e**
Penetrability (cm)	*7.93*	*0.75*	4.14	0.57
Phosphorus (mg/kg)	0.77	0.05	10.32	2.5
Organic carbon (%)	6.39	0.56	7.64	0.55
Potassium content (Cmol/kg)	0.65	0.11	0.73	0.08
Total Nitrogen	0.71	0.05	0.77	0.05
Temperature (°C)	18.82	0.52	19.19	0.72
Soil pH	*5.62*	*0.06*	5.62	0.06
Moisture content	1.96	0.17	2.46	0.32

Chapter 6 **Synthesis**

6.1 **Background**

Frugivorous vertebrates provide the bulk of seed dispersal services in tropical forest ecosystems, where between 70 and 94 % of tree species are estimated to rely on vertebrates for the dispersal of their seeds (Duncan and Chapman, 1999, Jordano, 2000, Wunderle, 1997). However, alarming rates of deforestation and degradation, especially in Afrotropical lowland and montane forests, have led to massive declines in vertebrate frugivore abundance and diversity, severely limiting the provision of dispersal services, and natural regeneration in these ecosystems (Kirika et al., 2008). This poses a serious threat to the future of these forests and the many valuable material, spiritual and ecological services that they provide to humans (Costanza et al., 1997, Price et al., 2011). While much laudable practical work is being done to conserve, and restore Africa's degraded forests (Barnes et al., 2014, Chapman and Chapman, 1999, Chapman, 2008, Chapman et al., 2010) and facilitate the recovery of declining vertebrate dispersers (Chapman et al., 2010), an essential corollary is research aimed at understanding, and applying the contributions of secondary or complementary dispersers from other animal taxa to forest regeneration. With declining population of vertebrate frugivores, ants could become more important as primary and secondary dispersers, especially of small diaspores that have been partly processed and dispersed by frugivorous birds (Christianini and Oliveira, 2010).

Evidence from research in Neotropical forests and savannas suggests that ants, which are a very diverse and abundant insect taxon, interact frequently with diaspores (seeds and fruits) primarily adapted for dispersal by vertebrates and contribute significantly to seed survival, germination success, and natural regeneration (Christianini et al., 2007, Gallegos et al., 2014, Pizo and Oliveira, 1999, Pizo et al., 2005). However, such interactions and their effects on diaspore fate and plant regeneration have not been explored in Afrotropical forests. Therefore, the main aim of this thesis was to investigate interactions between ants and non-myrmecochorous diaspores in Ngel Nyaki Forest reserve (NNFR) and determine whether they can contribute to natural regeneration in this important but degraded West African

montane forest. Specifically, I sought to identify ant species that interact with diaspores, factors that influence such interactions and whether such interactions result in dispersal and/or increased germination success. Dispersal of seeds by ants, to potentially safe sites even over small distances, could be an important contribution to diaspore survival and regeneration, given the high rates of seed predation often reported in African forests (Hart, 1995). This study represents the first documented research from the Afrotropics on the subject.

Because the ground-dwelling ant community in NNFR was hitherto unknown, I began my investigations by first sampling ant species richness and diversity within the Reserve.

6.2 Could lower ant species richness and diversity in montane forests limit ant-diaspore interactions?

This study provided the first baseline checklist of the ground dwelling ant community in NNFR and extends the records of several taxa. The list (Chapter 2), includes 11 of the 56 ant genera recorded in Nigeria, and notable, was the presence of *Pheidole megacephala*, known to be one of the most invasive ant species in many parts of the world (Hoffmann et al., 1999). Although no evidence was found that it might be invasive in this area (only a few individuals were encountered), it may be in the early stages of invasion and could potentially eliminate ant species that might be important diaspore dispersers. Overall ant species richness and diversity indices in NNFR were low, as revealed by the Shannon-Weiner index of 1.41 and the number of morpho-species (17). This could be the result of disturbance within this ecosystem, but an alternative explanation is the environmental constraint imposed by the high altitude (1600 m) of the Reserve, and would fit the pattern of lower ant richness and diversity found in high altitude montane forests as have been observed in East Africa (Fisher and Robertson, 2002), Malaysia (Brühl et al., 1999), and North America (Sanders, 2002). This pattern has been explained in terms of a reduction in ambient temperature and the associated reduction in productivity and changes in precipitation patterns (Brühl et al., 1999). Ant community within NNFR belong to either of two broad foraging guilds; generalist-omnivores or generalist carnivores. The two ant species, identified as *Pheidole* sp.1 and *Myrmicaria opaciventris* (both

Myrmicinae), observed interacting with diaspores were considered to be in the omnivorous-generalists group. The fact that ants in this guild exploit a wide variety of food sources, including plant and animal material (Hölldobler and Wilson, 1990) means that they would exploit available diaspores opportunistically within NNFR. Although both ant species had small body size (<0.2mm), they occupied different positions on the Principal component analysis (PCA) orthogonal plane indicating that they differed in functional traits. *Pheidole* sp.1 though smaller than *M. opaciventris* appeared to have longer mandible length relative to its body size.

In the context of beneficial ant-diaspore interactions, a possible implication of the low ant species richness in NNFR and perhaps other montane forests is that it could restrict the number of ant species potentially interacting in any beneficial way with diaspores, thereby limiting overall contribution to dispersal and regeneration. This may explain why only two species: *Myrmicaria opaciventris* and an unidentified *Pheidole* sp.1 were observed interacting with fallen plant diaspores during field surveys and diaspore removal experiments. This finding stands in stark contrast to the Neotropics where the number of ant species found interacting with non-myrmecochorous diaspores within a single study location/site may often reach up to 20 species (Christianini et al., 2007, Pizo and Oliveira, 1998). One study found 57 ant species interacting with diaspores of only one non-myrmecochorous plant, *Cabralea canjerana*, a Neotropical tree (Pizo and Oliveira, 1998). It must be stated though, that those sites in Neotropics were usually lowland rainforests of considerable size, which contain some of the most diverse and species-rich ant communities in the world (Hölldobler and Wilson, 1990).

6.3 Pattern of ant-diaspore interactions in NNFR

The ant-diaspore interactions recorded in NNFR did not appear specialised. *Pheidole* sp.1 and *Myrmicaria opaciventris* interacted with diaspores of 10 plant species; moving diaspores of six of the 13 plant species used in diaspore removal experiments and consuming fleshy appendages on-the-spot, from diaspores of four plant species. It is clear from these observations that ant-diaspore removals within NNFR may rely heavily on these ant species which were abundantly sampled in the forest, edge and grassland habitats. Moreover, these two species were overall the most abundant ant

species sampled, and this underscores the significance of the role they play in the removal, and possibly dispersal of diaspores within NNFR; especially in the forest edge and grassland habitats. *M. opaciventris* occurred mainly in the grassland and dominated the edge habitat, but it was absent from the core of the forest whereas *Pheidole* sp.1 was present and equally abundant in all three habitats.
Common ant species can provide the bulk of diaspore removal even for non-myrmecochorous diaspores (Barroso et al., 2013).

This study identified which diaspores ants move within NNFR and locations where they are deposited. However, the quantity and quality of diaspore removal by ants determines their effectiveness as seed dispersers (Graham et al., 1995, Schupp, 1993). This is important because if such removal is to aid plant recruitment and natural regeneration, substantial amounts of diaspores need to be moved and deposited in suitable microsites (Schupp et al., 2010). Since ants removed up to 55% of available *T. orientalis* diaspores within 24 hours (details in chapter 4), it suggests that ants can move a substantial number of diaspores over time.

The fact that diaspores of *T. orientalis* and *Z. leprieurii* were the most frequently moved shows that ants prefer diaspores of some species over others, although other morpho-chemical factors that influenced such preferences in this study were not clear. Diaspore size, however, stood out as a major factor influencing removal by ants; highlighting the importance of the traits of interacting partners. Because ants involved in diaspore interactions are small ants, they moved very small diaspores ranging from 0.01g – 0.24g, indicating that only plants that produce small diaspores can benefit from ant removal in NNFR. Tropical montane habitats harbour higher proportions of small diaspores compared to lowland forests where diaspores are much larger (Chapman et al., 2016) and studies in the Neotropics confirms findings from this study that size is a major morphological trait that strongly influences which diaspores ants interact with and how they do so (Gorb and Gorb, 1995, Pizo and Oliveira, 2001).

While diaspore removal was limited to small diaspores, both ants interacted with larger diaspores that had fleshy appendages, by consuming such appendages on the spot. Arils on the top of diaspores of *P. pinnata* were completely consumed by both

ant species, especially *Myrmicaria,* perhaps because this ant is slightly larger than *Pheidole* sp.1. and attended the diaspores in larger numbers. Germination experiments of cleaned seeds simulating ant seed cleaning indicated that cleaning provides faster germination. Cleaning thus appears to promote earlier germination, which may be beneficial for seeds to escape predators and unfavourable environmental conditions in the post-dispersal season (Bowers and Dooley, 1993, Christianini and Oliveira, 2010). This can lead to improved chances of successful recruitment (Orrock and Christopher, 2010). Seed cleaning in NNFR by *M. opaciventris* and *Pheidole* sp.1 can potentially minimize seed mortality and enhances germination and seedling recruitment. It's been shown elsewhere, that removal of fruit pulp from diaspores by ants reduces seed mortality resulting from pathogenic infestation (Brener and Silva, 1996, Ohkawara and Akino, 2005, Oliveira et al., 1995).

6.4 Diaspore removal by *Pheidole* sp.1 and *Myrmicaria opaciventris*: predation and/or accidental dispersal

The fate of diaspores removed by *Pheidole* sp. 1 and *M. opaciventris* in this study, was not established, and whether such removal is dispersal or predation could not be ascertained once diaspores had been deposited into nests or leaf litter. Untangling the benefits of diaspore removal by ants (dispersal) from the losses (predation) has always been difficult; especially in the case of non-myrmecochorous seeds that are not adapted for ant-dispersal (Arnan et al., 2012). Diaspore removal is either often misinterpreted as dispersal or predation (Lambert et al., 2005).

Although ants in this study exhibited limited dispersal distance (mean = 0.28 m; minimum = 0.01m; maximum = 1.2 m), a significant number of diaspores ended up in ant nests. And since piles of cleaned, intact seeds were not seen around ant nests, it is possible that most of the seeds were consumed while a few were discarded or abandoned unharmed within the nest.

Interactions observed between *M. opaciventris* and diaspores of *T. orientalis* were interesting in this regard. This ant only removed seeds of *T. orientalis* to its nest if they had some fruit pulp left on them, but it showed little interest in cleaned seeds. The few occasions when *M. opaciventris* removed cleaned seeds, seeds were always

dropped after a few centimetres. This behaviour is perhaps an indication that removal of *T. orientalis* diaspores (and other species) by *M. opaciventris*, results in dispersal rather than predation. Not much is known about the diet of this species, but diaspores seem to be not part of it (Kenne and Dejean, 1999, Kenne and Dejean^{o}, 1997).

In contrast to *M. opaciventris*, the interaction of *Pheidole* sp.1 with *T. orientalis* diaspores appears more likely to be predation rather than dispersal, as this ant moved significant numbers of diaspores of this plant to its nest regardless of whether they had fleshy appendages or not. Besides, it was never seen to drop such diaspores *en route* its nest, which indicates that the seed was its primary object of interest. Ants from the genus *Pheidole* are well known as important seed predators (Lambert et al., 2005, Retana et al., 2004, Vaz Ferreira et al., 2011). Nonetheless, because ants that prey on diaspores play dual roles as accidental dispersers (Beaumont et al., 2011, Hughes and Westoby, 1990, Predavec, 1997, Retana et al., 2004), *Pheidole* sp.1 may act in the same way and minimally impact recruitment of *T. orientalis*. About 14.5% of diaspores of *T. orientalis* observed being deposited in nests of both *Pheidole* sp.1, and *M. opaciventris* were seen germinating over four months.

6.5 Conclusion and future research

Forest regeneration is largely dependent on successful seed dispersal, germination and seedling recruitment. The decline in vertebrate seed dispersers in the Afrotropics resulting from anthropogenic degradation of forests means that smaller secondary dispersers, such as ants, could become more important in the dispersal of seeds adapted for vertebrate dispersal and contribute to plant recruitment and regeneration in degraded forests as evidenced in the Neotropics. This thesis has taken the first step in filling the massive knowledge gap regarding the nature of interactions between ants and non-myrmecochorous diaspores in Afrotropical forests- specifically, in an Afromontane forest. Ants in NNFR moved small diaspores albeit over short distances and deposited them in safer microsites, which can reduce loss to seed predators and desiccation on the forest floor. Seed cleaning, which can reduce diaspore mortality resulting from pathogenic infestation, and improve germination is likely to be the more common beneficial ant-diaspore interaction in this area dominated by two small myrmicine ant species. Compared to the Neotropics, my

results suggest that the influence of ants on the regeneration of non-myrmecochorous plant species in Afromontane forests is minimal although this is likely to be context specific and based on the traits of interacting ants and diaspores. Context-specific factors e.g. elevation, ant and plant species diversity and combinations, need to be investigated in other Afrotropical forests, and better methods developed to quantify post-dispersal seed fates accurately.

To adequately assess the impact of ants on seed dispersal and regeneration in NNFR, the quality of ant-diaspore dispersal needs to be better elucidated. To achieve this, the proportion of diaspores that germinate and become successfully recruited after ant handling needs to be determined for selected plant species. The fact that the ant species and plant species involved in these interactions have been identified provides baseline information for follow-up studies. Controlled experiments around the nest environment would clarify the fate of removed diaspores taken into ant nests. Determining whether ant-cleaning of diaspores offers the additional advantage of protection from pathogenic infestation of diaspores beyond removal of fruit pulp/aril would add to our understanding of the impact of ants on the viability of even diaspores too large for them to move and disperse. This is crucial because pathogenic infestation is known to be one of the main causes of seed mortality in tropical ecosystems (Augspurger, 1990, Oliveira et al., 1995).

In the wider context of understanding ant interactions with non-myrmecochorous plant species and the ecological consequences, I suggest a systematic review of current trends and hypotheses to summarise what is known so as to identify knowledge gaps that need to be addressed in future studies. In the Afrotropics where little is known about such interactions, more studies are needed and could test the proposed hypotheses:

- A decline in ant species richness and diversity at high altitudes would lead to fewer ant-diaspore interactions in montane forests compared to lowland forests.
- Because few ant species are involved in ant-diaspore interactions at high altitudes, there will be a tendency for keystone dispersers to emerge.

- Effective ant-diaspore dispersal in both lowland and montane Afrotropical forest will depend more on the presence of large ant species that forage individually and far from their nests.

Efforts to conserve Afromontane and lowland forests must not be relented to preserve vertebrates that are indispensable to long-distance seed dispersal and regeneration.

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General Appendix

Figure A: Seed and fruit of *Allophylus africanus*

Figure B: Seed and fruit of *Antidesma* sp.

Figure C: Seed and Fruit of *Paullina pinnata*

Figure D: Seed and Fruit of *Rauvolfia vomitoria*

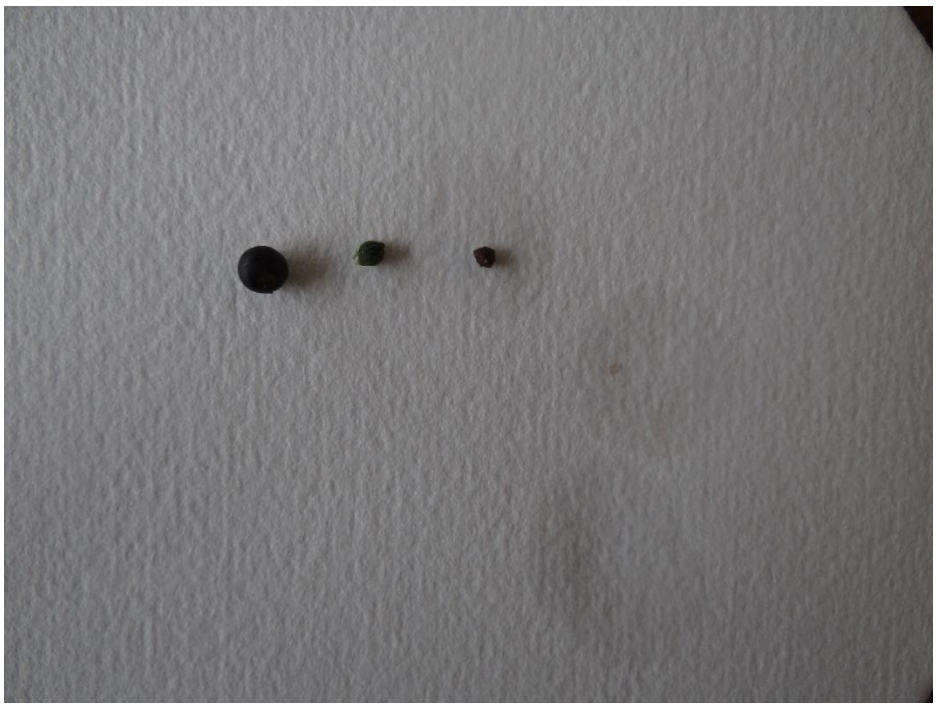

Figure E: Fruit, seed and cleaned seed of *Trema orientalis*

Figure F: *Myrmicaria opaciventris* cleaning fruits of *Psorospermun aurantiacum*

Figure G: Seeds of *Macaranga occidentalis*